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THE EFFECT OF GONADECTOMY ON BODY STRUCTURE AND BODY WEIGHT IN ALBINO RATS

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Many studies have been reported on the effect of gonadectomy on body weight and structure, but there is a lack of agreement in the findings.

In castrated male ablino rats, weight losses or smaller weight gains were noted by Evans and Simpson (1927) and Korenchevsky (1930, 1934), normal growth curves by Stotsenberg (1909), and larger gains in weight by Slonaker (1930). Slonaker however observed his animals over a longer period of time than other workers and subjected them to activity experiments. The determining factor in weight gain or loss was attributed by Van Wagenen (1928) to the age of the animal at the time of castration, and by Slonaker (1930) to the changes in activity.

In the female most of the workers (Stotsenberg, 1913; Hatai, 1913; Slonaker, 1930; Reed, Anderson and Mendel, 1934) have reported that oophorectomized animals gain more weight than controls. Schultz (1934) found this increase in only 35 per cent of his animals.

With regard to changes in body structure the reports are also conflicting. Stotsenberg (1913) attributed the increase to both accumulation of fat and generalized enlargement. Schultz (1934) states that even in the oophorectomized animals which have not gained weight the depot fat stores are increased and the liver fat is reduced. Others (Cramer and Marshall, 1923; Reed, Anderson and Mendel, 1932) have failed to find increased deposition of fat in oophorectomized rats. In male castrates a decrease in nitrogen chiefly due to the increased proportion of fat and solid matter was reported by Korenchevsky (1930) and Korenchevsky and Dennison (1930).

It seemed important to study again the effect of gonadectomy on body structure and to correlate these changes with food intake. In order to

measure more accurately the body constituents chemical analyses were made of the carcasses. Nitrogen balance studies also were made and x-ray plates of the skeleton were taken. It was expected that the studies would throw some light on the rôle of gonadectomy in the production of obesity.

METHODS. Fifty-four rats were used in the experiment, 26 males and 28 females. The females consisted of four litters of six each, and four rats of the same age and weight but not known litter mates. Four litters of six each, and two rats of similar age and weight but not known litter mates comprised the male group. One half of each group were gonadectomized and the other half used as controls.

Three males were castrated at 33 days of age, six at 56 days, three at 59 days and one at 73 days. The females were oophorectomized at the ages of 33, 59, and 73 days. On maturity, vaginal smears were made daily for a period of ten days following the method of Long and Evans (1922). The normal animals passed through the oestrous cycle but the operated animals did not.

The animals were kept in a separate room with plenty of ventilation and at as even a temperature as can be maintained without special equipment. One individual took entire charge of the animals, cleaning cages, weighing, and feeding. A special type of food container was used which made it possible to determine quantitatively the food intake. This was computed at weekly intervals. Individual fountain drinking cups were used.

A dog food (Dickinson's) in pellet form, ground before feeding, with a chemical analysis of protein 22 per cent, fat 4.5 per cent, carbohydrate 48.5 per cent, moisture 9.13 per cent, ash 9.5 per cent, calories 1.9 per gram, constituted the basis of the diet. Additional weekly rations of lettuce, carrots and oranges were given in equal quantities to all rats and in amounts which were completely consumed. The food was adequate in all vitamin and mineral requirements as shown by the growth curves which compare favorably with those reported in the literature (Donaldson, 1915; Stotsenberg, 1909, 1913; Slonaker, 1930).

Each animal was weighed on scales sensitive to a tenth of a gram at weekly intervals. In the charts the weight from birth to the beginning of the experiment was plotted as a straight line from four grams, the average birth weight of rats, to the first known weight.

For the nitrogen balance experiments a single animal was kept in a metabolism cage for a period of three days, and records were made of the beginning and end of the period. Nitrogen determinations were made in duplicate on the food, urine, feces by the macro-Kjeldahl method.

X-ray plates were taken of the animals placed on their backs with the extremities tied to a frame. The animals remained quiet but were not anesthetized. The films were taken at a distance of 28 inches, $\frac{1}{4}$ second exposure, 30 M.A. and 32 K.V.P. The measurements included the humerus, femur, tibia and pelvic bones.

For chemical analyses the rats were killed with ether, litter mates on the same day. The stomach and intestines were removed, the visceral fat trimmed off and added to the carcass, and the weight of the eviscerated animal recorded. The carcasses were preserved on the coils of a refrigerator until they were ground up in an ordinary meat grinder. The difference between the weight of hashed animal and the weight at death gave the loss by evaporation. A correction factor, $= \frac{\text{weight at death}}{\text{weight after grinding}}$, was calculated in each case and applied to the weights of all the samples taken. All samples were weighed out immediately after the grinding with determinations in duplicate.

The fat was determined by the Kumagawa-Luto method (Asada, 1923). The total purified petroleum ether extract after saponification was counted as fat. This represents the sum of the high molecular weight fatty acids and the unsaponifiable material. The latter was not separated as its quantity is small and the isolation takes some time.

Total nitrogen was estimated by the macro-Kjeldahl method; total solids by heating 10 to 15 grams of tissue for 48 hours at 100° to 110°, and the ash by incineration in a silica crucible to a constant weight in a muffle furnace at about 700°.

RESULTS. *Body weight, females.* All spayed females gained more weight than their litter mate controls. The two spayed rats of the same age but not known litter mates showed a similar smaller gain. The growth curve of the spayed animals was similar to that of the normals. The maximum difference in weight between the operated and unoperated female members of the same litter was reached between the twelfth and twentieth weeks. Following this the control group gained slightly more than the spayed rats but the operated group always maintained a greater weight.

Although the curve of weight gain in all spayed animals was similar, there was considerable difference in the maximum weight reached. The weight gains of the spayed animals in the three litters of the same age (fig. 1) also showed differences. Litters B and C reached a maximum of 218 grams when 28 weeks old while litter D, a similar group, reached only 186 grams. Litter D did not reach its maximum until the thirty-sixth week. This emphasizes the importance of comparing litter mates.

Three animals of litter G operated on when 59 days old were 40 grams heavier than the controls at the twentieth week. Following this the three controls gained slightly more than the spayed rats so that the difference averaged about 30 grams. Two spayed females of 73 days of age, not know, litter mates, failed to surpass the two controls until after the twelfth week. Following this a greater weight was maintained by the spayed animals. The graphs showing the growths of the individuals of litter G and four other animals not known litter mates are similar to those illustrated in figure 1 and are omitted for this reason.

In summary it may be stated that all female rats, when spayed between 33 and 79 days of age, gained more weight than their controls and maintained this difference in weight over a period of 52 weeks.

Body weight, males. The weight gain in males was variable. The animals (litter A) operated when 33 days old in July became heavier than the unoperated group after the fourth week. This increase was maintained for a period of 38 weeks but never exceeded 11.1 grams. The three animals in litter J (six in number) castrated when 59 days old (May) lagged behind the control group in the first four weeks, then gained more weight and maintained a greater weight for the duration of the experiment (58 weeks). At the end of 34 weeks the weights of the two groups were

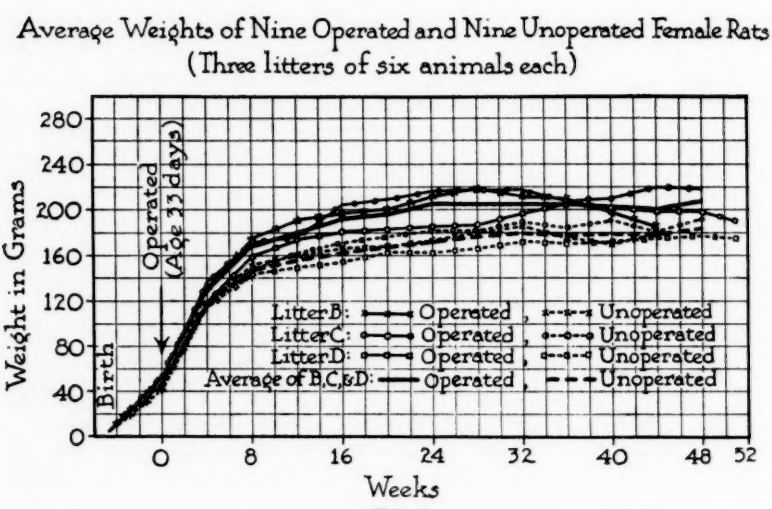

Fig. 1

the same. From the 34th to the 58th weeks the castrated animals again increased in weight more rapidly attaining a difference of 50 grams at end of 58th week. In both litters E and F (fig. 2) operated in July when 56 days old and controls gained and maintained more weight than the castrated animals. The weight difference was as great as 56.4 grams. Averaging all the animals of this group the weight gain ranged from 18.5 grams at the end of four weeks to 47.5 at the end of forty weeks in favor of the unoperated group. From a consideration of the behavior of these four litters it is evident that castration did not produce a clearly predictable alteration from the normal of the weight curve.

Food consumption. The spayed females in the four litter groups consumed more food than the unoperated when total grams of food eaten are

Average Weights of Six Operated and Six Unoperated Male Rats
(Two litters of six animals each)

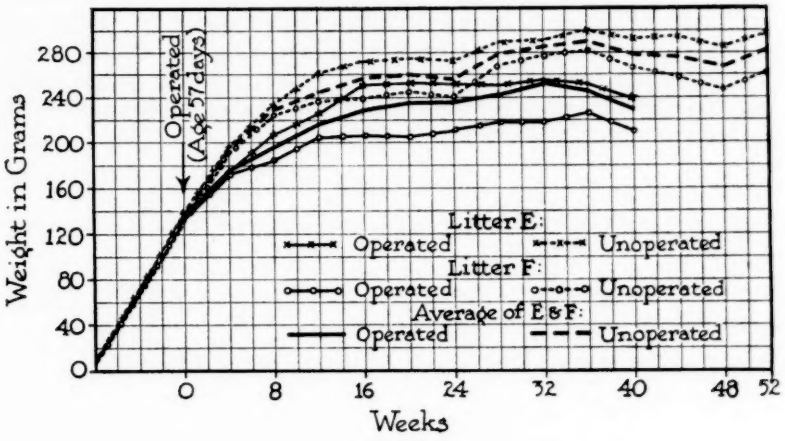

Fig. 2

Average Caloric Intake of Male and Female Rats

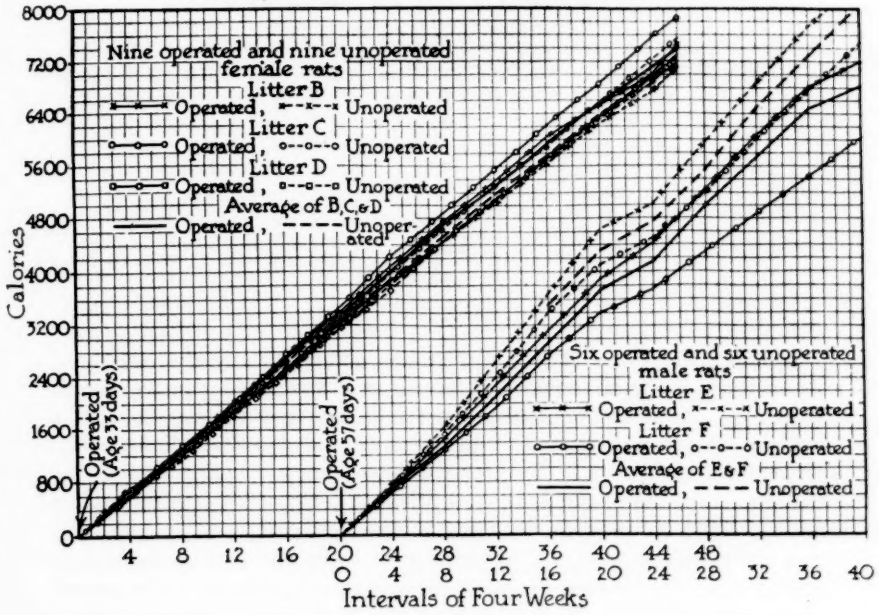

Fig. 3

considered. At the end of 44 weeks (fig. 3) litter B had eaten 105.2 grams more than their controls; C, 250.2 grams and D, 67.2 grams more. Expressed in calories the values are 199.8, 475, and 127.6. The average excess intake of the three litters was 77.9 grams or 159 calories. In litter G the spayed animals consumed similarly more food than the control group. The difference became progressively greater each four week period, reaching a total of 429 grams or 816.4 calories at the end of the 60 weeks of the experiment when the animals were 479 days old.

TABLE 1

Intake of food in grams per gram of body weight during each four week experimental period

4 WEEK PERIODS	FEMALES LITTER G		FEMALES LITTERS B, C, D		MALES LITTER A		MALES LITTERS E AND F		MALES LITTER J	
	O*	C†	O	C	O	C	O	C	O	C
No. of rats.....	3	3	9	9	3	3	6	6	3	3
1	2.0	2.3	2.5	2.7	2.3	2.3	2.1	2.2	1.9	2.0
2	1.7	2.0	2.1	2.2	2.1	2.1	1.6	1.6	1.8	1.9
3	1.6	1.6	2.0	2.2	1.9	1.9	1.5	1.5	1.9	2.0
4	1.7	1.9	1.9	2.1	1.8	1.8	1.6	1.7	1.9	2.0
5	1.7	1.9	1.7	2.0	1.4	1.4	1.8	1.8	1.7	1.6
6	1.8	1.9	1.6	1.9	1.4	1.5	1.4	1.7	0.8	0.8
7	1.6	1.7	1.7	1.9	1.5	1.6	1.6	1.3	1.4	1.6
8	1.5	1.6	1.7	1.8	1.6	1.5	1.3	1.5	1.2	1.6
9	1.5	1.7	1.5	1.7	1.6	1.4	1.6	1.5	1.3	1.5
10	1.5	1.7	1.5	1.8	1.4	1.4	1.5	1.1	0.8	1.4
11	1.4	1.4					1.5	1.5		
12	1.4	1.6					1.5	1.4		
13	1.4	1.5					1.4	1.3		
14	1.3	1.7					1.6	1.4		
15	1.6	1.8								

* Operated.
† Control.

In the males, litter A, the two groups ate practically the same. In litters E and F (fig. 3) the control animals, which weighed more, consumed more food. In a four week period at the beginning of the experiment the average difference amounted to 50 grams, by the close the difference had amounted to 760 grams. In litter J the food intake remained practically the same until the forty-fourth week when the castrates began consuming more food, which was consistent with their weight gain.

An increased amount of food would seem to be required by the spayed females until the food per gram of body weight is calculated. The spayed group utilized slightly less food to maintain their weight and gain during

each four week period (table 1). This difference persisted throughout the experimental period of 40 to 60 weeks.

In the castrated males despite the weight fluctuations, in which on the one hand the controls (litters E and F) gained more weight and on the other hand the operated animals (litter J) gained more weight, the food intake per gram of body weight remained approximately the same. In litter A where the weights were practically the same the food intake remained identical.

TABLE 2

Measurements of bones in millimeters from x-ray plates

	FEMALES						MALES	
	O*	C†	O	C	O	C	O	C
August 14, 1933								
Rat number	9	9a	6	6a	2	2A	7	7a
Femur	26	25	27	27	31.5	28	27.5	28
Tibia	33	31	32	31.5	36	33	34	32
Pelvis	36.5	32.5	34	34	39	36	37	35.5
Humerus	22	21	21	21	24	22	23	23
October 14, 1933								
Femur	29	26.5	28	29.5	31.5	29	32	31
Tibia	35	34	35.5	33.5	37	34	38	36
Pelvis	38	35	37.5	36	40	36.5	42	39.5
Humerus	24.5	22.5	23	23	24	22.5	26	25.5
January 26, 1934								
Femur	31	28	30	29.5	31.5	29	32	32
Tibia	37	34	36	36	37.5	35	39	37.5
Pelvis	39	35.5	39.5	38	41.5	36.5	42	42
Humerus	26	22.5	23	24	25.5	23	27	27

* Operated.
† Control.

Nitrogen balance. The nitrogen excretion of 28 animals, 12 males and 16 females, half operated and half controls, was determined. There seemed to be no tendency for the gonadectomized animals to store more nitrogen than the controls. Positive nitrogen balance proportional to weight gain and negative nitrogen balance in weight loss were found. The animals were necessarily confined to smaller cages than those to which they were accustomed. At times the weather was extremely warm, adding to the discomfort of the smaller cage. Several failed to gain weight, some lost

weight, and all consumed less food than they normally had been eating Since it was feared that a prolonged attempt to secure nitrogen balance experiments would interefere with the results of the fundamental growth experiments, they were discontinued. Therefore, the results are indicative rather than conclusive.

Skeletal meaurements from x-ray plates. Sumulong (1925) noted in male guinea pigs that the vertebral column and the long bones of the limbs of the castrates besides being somewhat heavier tend to grow longer than those of the controls. In the present experiment x-ray plates were taken of three spayed females and one castrated male and their litter mate controls. The first series was taken 40 and 102 days post-operatively, when the animals were 77 and 154 days old. The second series were taken two months later; the last series 204 and 257 days post-operatively at 242 and 309 days of age. The results are to be found in table 2. It will be noted that the operated females have longer bony structures. It is of course

TABLE 3

Average results of analyses

	FEMALES				
	Weight at death after evisceration	Per cent composition (grams per 100 grams fresh tissue)			
		Dry matter	N	Ash	Fat
Control rats (11)	164.7	35.35	3.48	4.22	9.52
Operated rats (11)	185.5	34.22	3.55	4.00	9.23

recognized that the number x-rayed is small, but yet the results are definite.

Chemical structure. In chemical analyses of the female rats the amount of dry matter, nitrogen, ash and fat was about the same in operated and control animals. The figures recorded (table 3) are significant only to the first decimal point and fall within the range for the normal as determined by Chanutin (1930) who analyzed 39 normal male and female rats. It is to be specifically noted that there was no increase in the total fat content. Any increase in weight of the spayed animals would therefore have to be attributed to a general increase in size rather than to a deposition of fat.

DISCUSSION. As previously stated, the object of this study was the evaluation of the rôle of gonadectomy in the production of obesity. The accumulation of fat in the body depots is possible only when the intake of food is in excess of the energy expenditure. When obesity exists, it is usually assumed that there has been either a decrease in voluntary activity or an increase in the appetite so that food beyond the requirements has

been eaten. In planning this experiment these factors were kept in mind and conditions were made as favorable as possible for the accumulation of weight. Thus the rats were given an excess of food and they had an opportunity at all times to eat to satisfaction. The activity of the rats was reduced by placing the animals in small cages, isolating them in a special room, having them cared for by the same individual and having food available to stop hunger restlessness as soon as it appeared.

Gonadectomy in the males caused no change in curves of body weight and did not influence the intake of food. Consequently, it was considered unnecessary to make chemical analyses of the male carcasses, and it was concluded that the removal of the testes was not an etiological factor in the production of obesity.

In the females gonadectomy was followed by an increased growth and not by an increased deposition of fat. Here again the removal of the ovaries could not be considered a factor in the production of obesity.

It is next of interest to consider this increased growth. It has been well established by a large number of experiments (Wang, 1923; Hoskins, 1925; Wang, Richter, Guttmacher, 1925; Slonaker, 1924, 1927; Richter, 1933) that gonadectomized animals, when placed in activity cages and given the opportuntiy to exercise, show much less activity than controls. Slonaker (1930) calculated the energy partition of normal and gonadectomized rats when they were actively exercising in revolving cages. The normals ate more food, exercised more, and therefore had less energy available for growth and basal metabolism than the ovariectomized animals. This is in sharp contrast to our results in which the total food consumed by the ovariectomized rat was larger than that eaten by the normal animal. If excess activity of the normals is responsible for an increased food intake, then under the conditions of our experiments there was no significant increase in the spontaneous activity of the normal animals over the gonadectomized litter mates. This agrees with the observations of one of us (H. H.) who was responsible for the feeding care of the animals.

The consumption of food over successive four week periods expressed in grams of food per gram of rat body weight (table 1) showed a somewhat lessened intake for the ovariectomized animals. However, the difference is not striking and if the activity of these animals was reduced by even a small amount, it can be seen that the energy required for a unit's increment in growth in the two classes of animals was identical. One then arrives at the conclusion that if the activity of a normal and an ovariectomized rat can be maintained at the same level, the food required for the growth expressed in calories per gram of body weight would be identical. The larger quantity of food eaten by the ovariectomized rats would represent additional food required by virtue of the growth.

The presence of the castration cells in the anterior lobe of the hypophysis

(Addison 1917), the prevention of the development of these cells by the injection of anterior pituitary hormone (Reese and co-workers, 1934), the increase in size of the pituitary after castration (Fischera, 1905; Korenchevsky, 1934), the greater activity of extracts from pituitary glands of castrated animals in gonad stimulating properties (Cushing, 1932) constitute evidence that castration definitely influences the gonadotropic properties of the pituitary.

With the confirmation of the evidence in the literature that ovariectomy results in increased weight, and with the further analysis that this weight increase is due to an increased growth, the conclusion seems warranted that ovariectomy influences also the growth promoting properties of the pituitary.

It is a speculative question as to whether this increased growth is to be attributed to a greater concentration of the growth hormone or to a facilitation of a normal quantity of the hormone. It would seem that the significance of the metabolic hormone of the anterior hypophysis, described by Anselmino and Hoffman (1931) and confirmed by Schultz (1934), is not sufficiently clear to warrant a discussion of its bearing on the present study.

SUMMARY

1. All spayed female rats gained and maintained greater weight than their controls.

2. The weights of males following castration varied. These variations are not predictable and are not significant.

3. The total food intake of the spayed females under the experimental conditions was greater than the normals, but the intake per gram of body weight was somewhat less.

4. There was no significant difference in the food intake of the castrated males and the normals.

5. The gonadectomized animals did not show an increased storage of nitrogen.

6. Slight increase in skeletal growth in gonadectomized females was demonstrated on x-ray examination.

7. The percentage of dry matter, nitrogen, ash, and fat in gonadectomized, eviscerated female carcasses, to which the visceral fat was added, falls within the normal ranges.

8. The increase in body weight in spayed animals is due to a general increase in size rather than to any increase in the deposition of fat.

9. Gonadectomy was not associated with an excessive appetite since the latter paralleled the growth process and at no time caused a deposition of fat.

10. Gonadectomy in the female influences favorably the growth process,

presumably through the growth hormone of the anterior lobe of the hypophysis.

11. Gonadectomy does not induce obesity in the albino rat.

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THE CIRCULATORY AND RESPIRATORY DISTURBANCES OF ACUTE COMPRESSED-AIR ILLNESS AND THE ADMINISTRATION OF OXYGEN AS A THERAPEUTIC MEASURE[1]

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Heller, Mager and von Schrötter (1900) found that rapid decompression of dogs from pressures of 60 to 70 pounds above atmospheric caused intravascular bubble formation which produced asphyxia by occlusion of the pulmonary capillary bed, and frequently paralysis of the hind extremities through a deprivation of the blood supply to the spinal cord. The object of the present investigation is to obtain further information regarding the causes of acute compressed-air illness and to ascertain the value of oxygen inhalation as a therapeutic measure.

The treatment of compressed-air illness in standard practice calls for recompression. Since the volume of an air embolus is inversely proportional to the barometric pressure upon it, recompression will reduce the size of the embolus and give relief to the characteristic symptoms. But unless the emboli are absorbed, then, when the pressure is again brought to normal, they will expand again and the symptoms will return. If time be permitted, air emboli will ultimately be absorbed and dissipated because the partial pressure of the nitrogen in them is higher than that of the nitrogen in the blood and body fluids, thus establishing a diffusion pressure head. The rate at which the absorption takes place is proportional to this pressure head.

In treating the compressed-air illness which was deliberately induced in our experiments, the dogs were recompressed at 30-pound gauge pressure, during which procedure some of the animals breathed air, while others breathed oxygen. When oxygen is breathed, the partial pressure of nitrogen in the arterial blood instantly becomes 0, and must approach 0 in the venous blood and tissues. As a result, the effective pressure head of the nitrogen in the embolus is much greater than when air is breathed. By a simple calculation, which cannot be discussed here, we have estimated that when air is breathed under a pressure of 30 pounds the partial

[1] This research was aided by the Miriam Smith Rand Fund.

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pressure of nitrogen in an air embolus is about 2144 mm., and in the arterial blood about 1773 mm., so that the nitrogen in an embolus has a pressure head of 2144 − 1773 = 371 mm. On the other hand, when oxygen is breathed this pressure head is 2144 − 0 = 2144 mm. Thus, under these conditions, oxygen inhalation should cause the absorption of air bubbles about 5.8 times as rapidly as air. Based upon such considerations, oxygen was administered in some of the experiments to ascertain its possible advantages in relieving the symptoms under investigation.

METHOD. Dogs were anesthetized with Dial.[3] One femoral artery was cannulated and connected to a manometer to record blood pressure. Samples of arterial blood were obtained from the other femoral artery. Samples of mixed venous blood were taken by means of a sound inserted through the external jugular vein into the right heart. Records were made of the respiratory rate, pulse rate, and arterial blood pressure. The oxygen saturation of the arterial and mixed venous blood was determined by equilibrating with air containing 25 per cent oxygen. When pure oxygen is breathed at a pressure of 30 pounds the capacity of the blood to hold oxygen is increased by approximately 4.2 volumes per cent, this being the amount taken up into physical solution. The carbon dioxide tension of the blood was determined by correlating the carbon dioxide content with the carbon dioxide dissociation curve. Bubble formation was observed macroscopically in the blood vessels of the living animal by a skin incision in the medial aspect of the thigh, and at death by autopsy.

The experiments were done in a pressure chamber large enough to accommodate the operators as well as the dog. The experimental procedure may, for convenience, be divided into 5 periods, and may be described in order of their sequence: 1. The control period, during which the dog breathed air at normal pressure and control records were made. 2. The compression period, during which the dog was subjected to air at 65-pound gauge pressure for 105 minutes. The pressure was then dropped to normal in 5 or 6 seconds. 3. The period which followed rapid decompression, during which the dog breathed normal air at normal pressure and the symptoms of compressed-air illness became apparent. Owing to the asphyxial character of these symptoms, which will be described later, this period is designated as the asphyxial period. 4. The recompression period, during which the pressure was raised to 30-pound gauge for 84 minutes followed by stage decompression for 30 minutes until the pressure was again normal. In some experiments air was breathed during this period, and in others oxygen was inhaled, in order to determine

[3] The authors wish to thank Dr. Charles C. Haskell of the Ciba Company, New York City, for his kindness in furnishing the dial-urethane solution used as the anesthetic in these experiments.

whether or not oxygen is more efficacious than air in dissipating air emboli. 5. The recovery period which followed recompression, and during which the dog breathed air at normal pressure. During this period the effects of oxygen inhalation, if any, were made apparent. Except in period 2, when the pressure was at 65 pounds, the operators were in the chamber with the dog.

TABLE 1

The blood pressure, respiratory rate and autopsy findings in dogs suffering from acute compressed-air illness

EXPERIMENT	BLOOD PRESSURE (MM. Hg)				RESPIRATORY RATE				AUTOPSY
	Control	Asphyxial period*	Recompression	Recovery†	Control	Asphyxial period*	Recompression	Recovery†	
Air inhalation									
2	147	80	108	82	15	28	8	10	Bubbles in right ventricle and in all blood vessels
4	97	68	70	84	30	62	20	40	Bubbles in all blood vessels
5	142	78	122	80	14	84	14	70	Bubbles in all blood vessels
6	115	54	100	100	26	100	20	80	Bubbles in all blood vessels
9	132	80	90	114	20	134	32	140	Bubbles in all blood vessels
12	146	64	118	100	30	84	56	86	Bubbles in right and left ventricles and in all blood vessels
Oxygen inhalation									
3	117	60	115		22	92	20		Bubbles in peripheral veins only
11	158	84	94	62	20	64	20	34	Bubbles in peripheral veins only
8	118	30	92	100	10	58	22	30	No bubbles
10	140	70	94	80					Bubbles in peripheral veins only
1	124	84	106	120	22	100	20	44	

* Data for asphyxial period taken just prior to recompression.

† Data for recovery period taken after breathing normal air for 1 hour.

EXPERIMENTAL RESULTS. The data on blood pressure, respiratory rate, and autopsy findings are given in table 1, and the data relative to the blood gases in table 2.

Asphyxial period. During the period which followed instantaneous decompression from a pressure of 65 pounds to atmospheric, and designated as the asphyxial period, the blood pressure at first rose sharply and then steadily fell. The duration of this period varied in different animals from 9 to 43 minutes, depending upon the rate at which the blood pressure fell. It was necessary in all cases to raise the barometric pressure again

TABLE 2

The oxygenation and the carbon dioxide tension of the blood of dogs suffering from acute compressed-air illness

EXPERIMENT	PERIOD*	OXYGEN CONTENT		ARTERIAL-VENOUS DIFFERENCE	OXYGEN CAPACITY	PER CENT OXYGEN SATURATION		PRESSURE CARBON DIOXIDE IN ARTERIAL BLOOD
		Arterial	Venous			Arterial	Venous	
	Air inhalation during recompression							
		volumes per cent						*mm. Hg*
6	Control		7.3		15.7			
	Asphyxial	6.8						
	Recompression	17.8	12.0	5.8				
	Recovery	10.5						
7	Control	19.3	15.2	4.1	22.4	86	68	
	Asphyxial	18.4	8.7	9.7				
	Recompression	25.8	11.5	14.3				
	Recovery	24.3	8.8	15.5	29.0	84	30	
9	Control	15.9	10.1	5.8	17.7	90	57	45.0
	Asphyxial	5.4	0.5	4.9	22.4	24	2	59.0
	Recompression	17.9	7.9	10.0	20.3	88	39	
	Recovery	5.9	2.3	3.6	22.8	26	10	
12	Control	14.6	12.0	2.6	15.9	92	75	38.0
	Asphyxial	6.9	2.8	4.1	18.7	37	15	51.0
	Recompression	16.0	11.3	4.7	16.8	95	70	
	Recovery	Death						
	Oxygen inhalation during recompression							
8	Control	20.9	16.7	4.2	23.1	91	72	37.0
	Asphyxial	23.5	17.1	6.4	26.7	88	64	46.0
	Recompression		20.5					
	Recovery	26.7	16.9	9.8	28.5	94	59	
10	Control	20.6	17.0	3.6	22.8	90	75	
	Asphyxial	14.6	7.7	6.9	26.1	56	30	
	Recompression	31.7	20.0	11.7	31.5†	100	64	
	Recovery	26.9	7.3	19.6	29.8	90	24	
11	Control	19.3	14.6	4.7	22.2	87	66	50.0
	Asphyxial	18.3	10.7	7.6	26.7	70	40	60.0
	Recompression	29.0	15.9	12.1	29.6†	95	54	
	Recovery	22.0	11.4	10.6	24.5	90	47	

* Data for asphyxial period taken just prior to recompression. Data for recovery period taken after breathing normal air for 1 hour.

† Four and two-tenths volumes per cent added to normal capacity by oxygen in physical solution.

to prevent death from circulatory failure. The falling blood pressure was accompanied by an accelerated respiratory rate, which increased from an average control value of 21 to 81. The pulse rate fell from an average of 138 to 94.

In every case, with the exception of experiment 8, the oxygen saturation of the arterial blood fell below 70 per cent, and in experiment 9 fell to 24 per cent. The deficient oxygenation of the arterial blood was accompanied by a rise in the arterial carbon dioxide tension. There was a marked increase in the arterial-venous oxygen difference, indicating a reduced circulation rate. Bubbles were frequently present in the blood samples drawn from the femoral artery and from the right heart. Small bubbles were seen in all cases circulating through the cutaneous arteries and veins which had been exposed by an incision in the thigh.

The picture here presented is one of acute anoxemia, caused by the formation of air bubbles throughout the entire vascular system, sufficient in volume to interrupt the continuity of the blood stream.

Recompression. During recompression at 30 pounds, or 3 atmospheres absolute, the volume of the bubbles is reduced to one-third of that existing at 1 atmosphere of pressure. The blood pressure improved but never returned to its control value, the average value of the latter being 130 mm. Hg as compared with 101 mm. under recompression; the respiratory rate decreased to the initial level; and the arterial blood became normally saturated. The arterial-venous oxygen difference, on the other hand, remained high. The beneficial effects of oxygen inhalation as compared with air in promoting the absorption of bubbles were not apparent during this period. The continued low blood pressure, notwithstanding a reduction of the nitrogen emboli to one-third of their initial volume, suggests a more or less sustained impairment of the vascular system caused by the profuse evolution of nitrogen into the blood stream which took place during the asphyxial period.

Recovery. It was found that the lowered blood pressure and increased arterial-venous oxygen difference which had failed to return to normal during recompression remained abnormal during the recovery period, irrespective of whether air or oxygen at 1 atmosphere was breathed. The respiratory rate of the dogs that breathed oxygen during the recompression period was much lower during the recovery period than that of the dogs which breathed air, the average values being 36 for the former as compared with 71 for the latter. The oxygen saturation of the arterial blood of the dogs which had previously breathed oxygen was normal during the recovery period, whereas the oxygen unsaturation of the dogs which had breathed air was very marked. Although figures for the per cent saturation are lacking in experiment 6, an oxygen content of only 10.5 volumes per cent indicates a very low saturation value. In experiment 7, the saturation was 84 per cent and in experiment 9, 26 per cent. In

experiment 12 the dog died soon after the pressure reached normal, but the dark color of the arterial blood left no doubt as to its oxygen unsaturation.

Autopsy. At autopsy it was found that the dogs which had previously breathed oxygen suffered from nitrogen emboli only to a minor degree, the only bubbles observed being confined to the peripheral vessels. The larger vessels and heart chambers were wholly free from bubbles. The presence of bubbles in the peripheral vessels may be due to the fact that the blood pressure was too depressed to overcome the inertia and force them into the general circulation where they would be absorbed. On the other hand, when the dogs had breathed air during recompression, not only were bubbles seen in the peripheral vessels but also in the venae cavae, the larger arteries and in both chambers of the heart. The right ventricle was frequently dilated and filled with a gas-blood emulsion and bubbles were present in the coronary arteries.

Discussion. Binger, Brow and Branch (1924) showed that when multiple emboli of the pulmonary vessels were produced in dogs experimentally by the intravenous injection of seeds of various sizes, rapid breathing and arterial anoxemia resulted. The rapid breathing was proved to be the result of anoxemia. With relief of anoxemia by oxygen inhalation rapid breathing ceased. The partial blockage of the pulmonary capillary bed by nitrogen emboli causes the blood to be shunted through that part of the route which offers the least resistance to blood flow. Binger, Brow and Branch found that, notwithstanding the reduction in the functional capillary bed of the lungs following embolic obstruction, the cardiac output per minute was only 14 per cent less than normal. They concluded that this was made possible by a dilatation of the intact route, accompanied by an increased pulmonary blood pressure. The normal saturation of the hemoglobin with oxygen is, therefore, obstructed in a twofold manner: 1, the increased rate of flow through the intact capillaries does not permit sufficient time for the blood to assume its normal load of oxygen; and 2, the dilated capillaries are crowded with corpuscles in columns so thick as to interfere with the normal inward diffusion of oxygen. Rapid breathing follows as a natural consequence of the anoxemia. The increase in the carbon dioxide content of the arterial blood is insignificant and by no means comparable to the oxygen unsaturation. Binger, Brow and Branch attribute this to the fact that the solubility of carbon dioxide is very much greater than that of oxygen.

The symptoms which follow the obstruction of the pulmonary circulation by the intravenous injection of particulate matter are so similar to the symptoms observed in acute compressed-air illness as to leave little doubt of the cause of anoxemia which follows rapid decompression from high air pressures.

In the experiments of Binger, Brow and Branch the blood pressure was

unaffected by pulmonary embolism, whereas in our own experiments the blood pressure fell and never returned to normal, even after the emboli had been apparently absorbed, or at least reduced to insignificant proportions. This difference in the reactions produced may be caused by a difference in the distribution of the emboli. Emboli produced by intravenous injection stop in the lungs and probably do not pass through into the general circulation, so that obstruction is confined to the pulmonary vessels only. On the other hand, air emboli produced by decompression are formed in the blood stream of the body as a whole and may, therefore, cause impairment of the bodily functions by interfering with the blood supply to the nervous tissues. Such impairment of the nervous tissue controlling the circulation may account for the depressed blood pressure associated with profuse air emboli caused by rapid decompression.

That the damage done to the nervous system persists even after the cause has been removed is indicated by the fact that the blood pressure, which is depressed while the barometric pressure is at 3 atmospheres absolute (30 lb.), remains unaffected when the barometric pressure drops to 1 atmosphere again. If the depressed blood pressure, existing under a pressure of 3 atmospheres, was caused by residual bubbles which failed to be absorbed, then, at 1 atmosphere of pressure, the bubbles should expand to 3 times their former size and the resultant injury to the nervous tissue should be increased proportionately. This, however, is not the case.

Further evidence of the injury to the nervous system, caused by emboli formed within the body, is afforded by experiments done in this laboratory upon intact unanesthetized dogs, which have been subjected to conditions of pressure and treatment identical with those described in this paper. These animals, after being released from compression, become paralyzed in the hind legs, and fail to recover even after a period of days. The paralysis which is manifest in the intact animals but obscured in those under anesthesia, leaves no doubt of the injury done to the nervous tissue.

Owing to the toxic effects of breathing oxygen under pressure the time of exposure is limited. It has been shown by Behnke, Forbes and Motley (1935) that pure oxygen under a gauge pressure of 30 pounds can be tolerated for about 3 hours before the toxic effects are manifested by contraction of the visual field, rise in blood pressure and increase in pulse rate. Behnke, Johnson, Poppen and Motley (1935) have shown that when the oxygen pressure is increased to 45 pounds syncope or a convulsive seizure may occur. It would, therefore, be wise to limit the pressure of the oxygen breathed to 30 pounds. At this pressure there can be little doubt that all, or nearly all, the nitrogen bubbles will be absorbed in severe cases of compressed-air illness.

SUMMARY

1. Nitrogen emboli were produced in the blood of anesthetized dogs by rapid decompression from air compressed to 65 pound gauge pressure for 105 minutes. The condition thus produced was similar to that of acute compressed-air illness.

2. The characteristic symptoms observed were rapid breathing, temporary rise followed by a fall in blood pressure, a retarded pulse rate, oxygen unsaturation of the arterial blood and a marked increase in the arterial-venous difference.

3. The rapid breathing and oxygen unsaturation of the arterial blood are attributed to embolic blockage of the pulmonary circulation.

4. The fall in blood pressure and increased arterial-venous difference is attributed to embolic injury to the nerve tissue which controls circulation.

5. In order to dissipate the emboli the dogs were recompressed to 30-pound gauge pressure. During this time some of the dogs breathed air and others pure oxygen, to test the efficacy of oxygen therapy in the treatment of compressed-air illness.

6. When the pressure again returned to normal it was observed that when oxygen was breathed, the oxygen unsaturation of the arterial blood was relieved, the respiratory rate returned to a normal value and only a few bubbles remained at autopsy. When air was breathed during recompression, a return to normal pressure caused the reformation of bubbles as observed at autopsy, and a return of the rapid breathing and oxygen unsaturation of the arterial blood.

7. The experimental results justify the use of oxygen to accelerate the absorption of nitrogen emboli.

The authors wish to acknowledge their indebtedness to The Linde Air Products Company, New York City, for the oxygen used in these experiments.

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THE MALTASE ACTIVITY OF THE BLOOD SERUM OF VARIOUS SPECIES

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It is generally agreed (Pringsheim, 1932) that pancreatic, salivary and malt amylases produce maltose as their end product. It has been assumed that they behave similarly when glycogen instead of starch is the substrate and that the glycogenases of various body tissues act in the same manner. Barbour's (1929) experiments disturbed this view for muscle glycogenase but the recent work of Carruthers (1935) supports the old idea in proving that maltose is the main end product of glycogen hydrolysis by the enzymes of both muscle and liver. The presence and distribution of maltase in the organism then has considerable importance. In attempting to find an explanation for the peculiar differences in the behavior of glycogen intravenously in relieving insulin shock in various species our interest was aroused in the in vitro maltase activity of various blood sera. The chief information on this subject resides in two investigations in which the authors reach opposite conclusions regarding the maltase activity of the serum in three species, namely, the rat, sheep and ox.

EXPERIMENTAL. We have examined the maltase activity of the serum of a number of species. Ox, sheep and pig bloods were obtained at the abattoir. Chicken and pigeon bloods were collected by decapitation of the birds. Rabbit, dog, monkey and cat bloods were taken by heart puncture. Mule, horse and goat blood specimens were drawn from superficial veins. Rat, guinea pig and mouse bloods were collected by exsanguinating the anesthetized animals through the severed abdominal aorta. The serum specimens were prepared at once and kept in the ice box until used, never more than two days.

We were interested in the maltase activity of blood serum as drawn and it should be pointed out that our results may be different from the actual maltase concentration. The reason for this is that like vegetable maltase the serum maltase works best in an acid medium and the optimum enzyme action is at 55°C. (Compton, 1924). We did not alter the pH of our sera and incubated them all at 37.5°C.

As a substrate a 1 per cent solution of maltose was used. A portion of

this was mixed with an equal volume of the serum to be tested and incubated at 37.5°C. The reducing action was measured at the beginning

TABLE 1

SPECIES	REDUCING POWER OF MALTOSE-SERUM MIXTURE AS GLUCOSE, PER CENT					INCREASE IN REDUCING POWER OF MIXTURE AS GLUCOSE, PER CENT				MALTASE ACTIVITY OF BLOOD SERUM		
Hours	0	2	4	8	16	2	4	8	16	Present study	Hynd and MacFarlane (1927)	Compton (1921)
Man	0.46	0.47	0.39		0.45	0.01	−0.07		−0.01	−		−
Monkey (Macacus rhesus)	0.44	0.43	0.44		0.37	−0.01	0.00		−0.07	−		−
Cat:												
Male	0.68	0.70	0.75		0.82	0.02	0.07		0.14			
Female	0.64	0.74	0.74		0.78	0.10	0.10		0.14	±	−	−
Female	0.35	0.49			0.49	0.14			0.14			
Rabbit:												
Female	0.60		0.62	0.63	0.63		0.02	0.03	0.03	−	−	−
Male	0.59		0.58	0.60	0.60		−0.01	0.01	0.01			
Guinea pig:												
2 females	0.58	0.59	0.57		0.54	0.01	−0.01		−0.04	−	−	−
2 males	0.61		0.61	0.62	0.68		0.00	0.01	0.07			
Chicken:												
Female	0.64	0.63	0.68		0.66	−0.01	0.04		0.02	−		
Male	0.77	0.77	0.76		0.79	0.00	−0.01		0.02			
Pigeon (10 birds)	0.76	0.72	0.93		0.70	−0.04	0.17		−0.06	−		
Mouse:												
25 albinos	0.71	0.89	0.92		1.12	0.18	0.21		0.41	+	−	
12 colored	0.55				1.07				0.52			
Rat:												
8 hooded	0.54		1.00	1.05	1.10		0.46	0.51	0.56	+	−	+
10 albinos	0.54		0.95	0.93	1.04		0.41	0.39	0.50			
Ox	0.50		0.88	0.94	1.00		0.38	0.44	0.50	+	−	+
Horse:												
Mare	0.46	0.78	0.89			0.32	0.43			+		+
Mule	0.48	0.84	0.69		0.81	0.35	0.21		0.32			
Sheep:												
Ewe	0.50	0.75	0.87		1.00	0.25	0.37		0.50			
Ewe	0.51	0.79	0.94		1.00	0.28	0.43		0.49	+	−	+
Goat	0.43	0.65	0.72		0.73	0.22	0.29		0.40	+		+
Dog:												
Male	0.51	0.91	1.02		1.04	0.40	0.51		0.53	+		+
Male	0.51	0.92	0.98		1.06	0.41	0.47		0.55			
Pig	0.44	0.99	1.06			0.55	0.62			+	+	+

of incubation and at periods varying from 2 to 16 hours thereafter. An increase in the reducing value indicated maltase activity. Toluol was

used as a preservative and controls indicated that there were no significant changes in the reduction of the serum reducing substances due to incubation. The sugar method of Shaffer and Somogyi (1933) was used to measure reducing values.

The results comprise table 1. With one exception we confirm Compton's (1921, 1923, 1924) experiments. Cat serum was found by Compton to belong in the negative group for maltase activity while we found in three separate specimens that it has a slight but measurable maltase action. Differing from Hynd and MacFarlane (1927) we agree with Compton in placing the rat, ox and sheep in the maltase positive group and also find that the mouse has maltase positive serum. Hynd and MacFarlane found mouse serum to be devoid of maltase activity and Compton did not examine it.

Discussion. There does not appear to be any dietary or zoological grouping of various animals by which they may be classed as to whether their serum is maltase negative or maltase positive. It is probable that the negative or positive activity of their serum in this respect has little or no relation to glycogen hydrolysis in their body economy, a process governed by cell enzymes which may bear no constant relation to those in the blood.

In light of the species differences in the maltase activity of the serum it is possible to explain the apparently diverse findings which initiated our interest in the subject. Noble and Macleod (1923) found that neither maltose nor glycogen relieved insulin hypoglycemia symptoms in rabbits. This might be expected for the blood sera of rabbits exhibits no maltase activity. Bollman, Mann and Magath on the other hand found (1925) that glycogen was quite efficacious in overcoming the hypoglycemia both after hepatectomy and from insulin injections in dogs. Since the dog belongs to the positive maltase group this is the reasonable result. Then Herring, Irvine and Macleod (1924) have shown that maltose is almost as efficient as glucose in alleviating the symptoms caused by insulin in the mouse and we have found contrary to Hynd and MacFarlane (1927) that the mouse also is in the positive maltase group.

SUMMARY

The blood serums of the pig, rat, dog, horse, ox, sheep, goat and mouse show positive maltase activity.

The blood serums of man, monkey, rabbit, guinea pig, chicken and pigeon are devoid of measurable maltase activity.

The blood serum of the cat has a small but probably positive maltase activity.

The maltase activity of the serum explains why neither maltose nor glycogen will relieve insulin hypoglycemia in rabbits, why glycogen is

efficacious in overcoming the hypoglycemia both from insulin and hepatectomy in dogs and why maltose will alleviate insulin symptoms in mice.

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THE RESPONSE OF THE RABBIT TO INSULIN

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The problem of the standardization of insulin has always been a difficult one due to the great variation both in the responses of different rabbits to a given dose, and of a given individual to repeated injections of similar doses of insulin. It is the purpose of this investigation to study some factors that may be involved in the response of the rabbit to insulin.

The immediate hyperglucemia which frequently follows the injection of commercial insulin preparations and which precedes the characteristic hypoglucemia has been the subject of many investigations. An excellent review of the subject has been made by Louise Hennequin (1). Rathery, Kourilsky and Laurent (2) working with normal dogs reported a frequent but not constant hyperglucemia produced by injections of commercial insulin preparations. Geiling and DeLawder (3) concluded that the extent and duration of the transient hyperglucemia produced by commercial insulin preparations is dependent on a sugar raising substance in the insulin, on the dose and on the individual susceptibility of the animals.

Whatever the mechanism may be, it has been our experience that with a given sample of insulin certain individuals showed this hyperglucemia, while in others, the onset of hypoglucemia was delayed, though there was no indication of hyperglucemia, and in still others, the onset of hypoglucemia seems to begin immediately after the administration of insulin.

Two different samples of insulin agreed in giving all of these reactions. Inasmuch as either sample of insulin produced all of the above responses, it was necessary to look for the reason in some peculiarity of the subjects which could be correlated with the characteristic behavior of the blood sugar rather than in the insulin. The following observations are offered as evidence that the presence and extent of an initial hyperglucemia is related to the sensitivity of the individual to insulin. Sensitivity was measured by the presence or absence of convulsions, and when these occur, by the time elapsing between the injection and their onset.

Rabbits were used as subjects. They were selected only with regard to physical condition. The animals were fed on a mixed diet of hay, oats, alfalfa and molasses. They received carrots twice a week and water

on the other five days. This diet maintained a normal growth of immature rabbits, and a full maintenance of weight in all fully grown adults when the animals were not subjected to experimental use. Any deviation from the normal behavior could, therefore, be attributed to either a direct or an indirect effect of the insulin administration. The animals were kept at least two weeks on the regular regimen before they were used in experimental work. They were confined in individual cages, 12 by 16 by 20 inches, during the period of observation in the laboratory and while in the animal room.

An inanition period of about twenty-four hours preceded the insulin injection. The blood samples were obtained from the marginal ear vein after hyperemia was produced by the application of a small quantity of xylene.

The total reducing substance of the blood (blood sugar) was determined by Somogyi's (4) micro-modification of the Shaffer-Hartmann method, using Somogyi's alkaline-copper-iodine reagent no. 1 described by Peter and Van Slyke (5).

The insulin used was the product of Eli Lilly and Co., known as "Iletin." During the course of the experimentation the insulin was kept in a refrigerator except for the time it was in actual use in the laboratory. The insulin was diluted with sterile 0.9 per cent sodium chloride, so that 1 cc. of the resulting mixture contained two units, the amount of insulin to be injected per kilogram of body weight. The injections were made subcutaneously in the region of the flank or the abdomen. The injection was followed by a mild massage.

The rabbits were injected, immediately after the onset of convulsions, with 5 cc. portions of 10 per cent sterile glucose. The animals were kept under observation for 6 hours, following the insulin injection, as our work indicated that the probability of convulsions occurring, after this time interval, was small with injections of two units of insulin per kilogram of body weight.

In measuring precision, the mean deviation $\epsilon = \sqrt{\frac{\epsilon d^2}{N-1}}$ rather than the standard deviation was used, for the reasons outlined by Scott (6). When the means of different series are compared, the mean deviation of the mean $\epsilon_M = \frac{\epsilon}{\sqrt{N}}$ was employed. For the mean deviation of quotients the following formula was employed;

$$\epsilon \frac{B}{A} = \frac{\sqrt{\left(\frac{B\epsilon_A}{A}\right)^2 + (\epsilon_B)^2}}{A}$$

The initial blood sugar was determined, two units of insulin per kilogram of body weight were injected subcutaneously, blood samples were

then taken at five minute intervals for thirty minutes, then at 45, 60, 90 and 120 minutes after the injection. The time of the onset of convulsions was recorded in each case.

According to their reactions, the animals were divided into the four following groups:

Group A. This group consists of 69 experiments, in which the rabbits showed no convulsions in 6 hours.

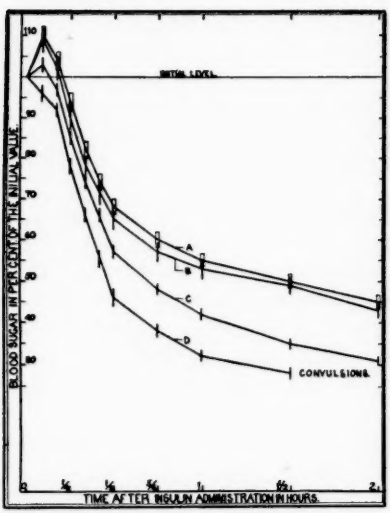

Fig. 1

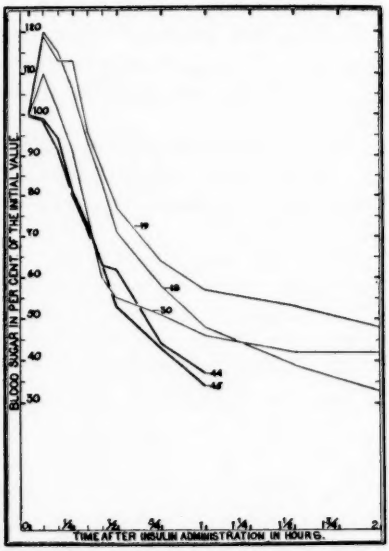

Fig. 2

Fig. 1. Time curves for the four groups of rabbits. The vertical lines indicate the precision with which the several points were established. The length of the lines represents ϵ_M.

Fig. 2. Time curves for different individuals. The curve for each animal is the average of 4 observations. Rabbits 18, 19 and 30 are very resistant to insulin. Rabbits 44 and 45 are very sensitive to insulin.

Group B. In this group, the rabbits went into convulsions from 181 to 360 minutes after the insulin was injected. In all there were 37 experiments.

Group C. In this group, the rabbits went into convulsions from 101 to 180 minutes after the insulin was injected. There were 66 experiments in this group.

Group D. In this group, the rabbits went into convulsions within

100 minutes after the insulin injection. There were 36 experiments in this group.

Figure 1 shows the relationship between the percentage change in the blood sugar and the time after the insulin injection.

It will be noted that the differences in glucemia, which have become established between the several groups ten minutes after the insulin was administered, are maintained through the period of rapid loss of sugar from the blood. The curves covering this period are therefore parallel.

The relationship between the initial insulin hyperglucemia and the sensitivity of the animal to insulin is well brought out in the individual animal. Figure 2 shows the time curve for different individuals.

As an animal changes in its sensitivity to insulin, its immediate insulin response changes accordingly. Figure 3 shows the changes in the time curves of the individual as the animal changes in its response to insulin. Only one sample of insulin was used on an individual rabbit.

In a control series, nineteen rabbits, which had uniformly given an immediate hyperglucemia following insulin injection, were injected with 1 cc. of 0.9 per cent sodium chloride per kilogram of body weight. The rabbits' initial blood sugars were determined. They were then injected with saline solution and blood samples taken at 5, 10 and 15 minutes after the injection. The following results were obtained:

	MGM. PER 100 CC.	ϵ	ϵ_M
Initial blood sugar	107	10.2	2.3
Blood sugar after injection:			
5 minutes after	107	10.8	2.5
10 minutes after	107	11.8	2.7
15 minutes after	109	11.1	2.5

The above results show that the handling of the rabbits and the injections, per se, do not have a noticeable effect on the blood sugar. They eliminate the possibility of the initial hyperglucemia being produced by the liberation of adrenaline or other hyperglucemic factor due to excitement in the animals.

The initial hyperglucemia may be produced either by the action of insulin or by some other substance present in commercial preparations of insulin which may act directly on the liver, or through the sympathetic-adrenal mechanism.

Ionesco, Cosmulesco and Tomesco (7) obtained the initial hyperglucemia after bilateral section of the splanchnics and after paralysis of the nerve endings of the sympathetic by ergotamine. They interpret these observations to mean that insulin produced the initial hyperglucemia

by direct action on the liver cells. Rathery, Kourilsky and Gilbert (8) obtained an initial hyperglucemia in a dog before and after adrenalectomy.

The fact that the hyperglucemia is produced in an animal after the removal of the adrenals, does not eliminate the possibility of the adrenals playing a part in the normal animal. Houssay and DiBenedetto (9) showed that the hyperglucemic action of extracts of the posterior hypophysis is reinforced by adrenaline, and is lowered by cutting the splanchnics. It is possible that the hyperglucemic action of insulin in the adrenalectomized animals is not a direct action on the liver but an indirect

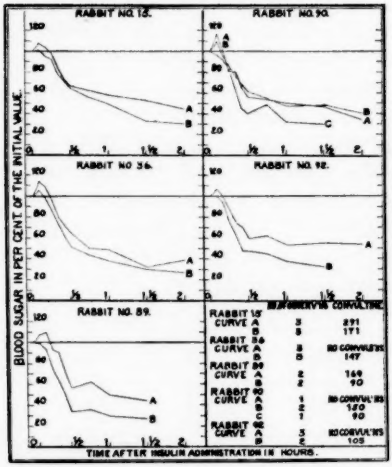

Fig. 3

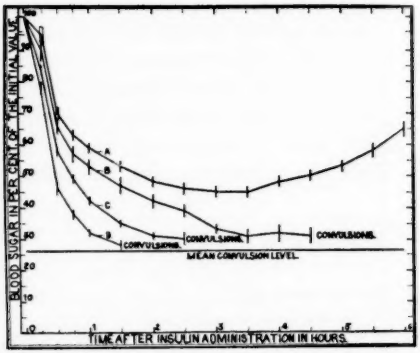

Fig 4

Fig. 3. Changes in the time curves of the individual as the animal changes in its response to insulin injection.

Fig. 4. Time curves for the four groups of rabbits. The vertical lines represent the precision with which the several points were established. The length of the lines represents $\pm \epsilon_M$. The mean convulsive level represents the non-fermentable fraction of the blood. At this level the true sugar is zero (Dotti, 11).

action through the hypophysis. In the normal animal it may be the sum of the action of both glands.

Geiling and DeLawder (3) claim that the initial hyperglucemia following the intravenous injection of commercial preparation of insulin is due to impurities present since crystalline insulin does not cause the primary rise of blood sugar when similarly administered.

Hennequin (1) maintains that the hyperglucemic substances could be present as such in the pancreatic tissue, or may be produced by the action of the chemicals, used in the preparation of insulin, on the glandular

tissue of the pancreas. It is necessary to admit the possibility of the existence in the pancreas of an antagonistic substance to insulin with specific hyperglucemic action.

Whatever the mechanism of the insulin hyperglucemia may be, it offers a means of determining the sensitivity of an animal to insulin, without subjecting it to an insulin convulsion. This is an advantage as it prevents possible injury to the animal. Thus the sensitivity may be determined if the rabbit is bled for the initial value, then injected with insulin and the blood sugar samples taken every 5 minutes for 15 minutes after the injection. After the blood samples have been taken, the rabbit may be injected with glucose and given food to prevent convulsions. From the blood sugar curve, the rabbit's convulsive response to insulin can be predicted.

Time curves were also obtained until the incidence of convulsions or for 6 hours after the insulin injection. The rabbits were bled for the initial sample, then injected with 2 units of insulin per kilogram of body weight, and blood samples taken every 15 minutes for the first hour and then every half hour until the animal went into convulsions or for 6 hours.

The rabbits were divided into 4 groups in the same manner as was described on page 540.

Group A consists of 59 observations
Group B consists of 28 observations
Group C consists of 59 observations
Group D consists of 36 observations

These results are shown in figure 4. Inspection of this chart shows that the curves may be divided naturally into three regions as follows:

1. The first 15 minute period. As was shown in the first figure, the hyperglucemia, in no case, outlasted the second blood sample, that is 10 minutes after the insulin injection. In every case the descending curve had crossed the initial level before the 15 minute sample was taken. In this group, the first post injection sample was taken after 15 minutes and as would be expected a hypoglucemia is shown in every curve. The degree of hypoglucemia is, however, influenced by the amount of hyperglucemia which can be presumed to have been induced and so the slope of the curves become progressively greater as the animals are more sensitive to insulin.

2. Following this initial period there is a period of about 15 minutes, shown better in the first figure, in which the curves are parallel, that is, in all groups sugar is leaving the blood at the same rate. It was at the end of this period that Scott and Dotti (10) drew the blood samples in their work on the assay of insulin. In this way they were assured of the maximum effect of the insulin dosage which would permit exclusion of the irregularities of the final region of the curves.

3. The final portion of the curve extends from the end of the period of uniform loss of sugar to the end of the period of observation. In all groups, the rate of sugar loss is slower in this portion of the curve than during the second phase, but aside from this the curves differ quite markedly. In the least sensitive animals, the rate of loss is rapidly decreased until it becomes nil and the curve takes a definite and constant upturn towards the initial sugar concentration. In the most sensitive animals, on the other hand, the sugar loss, though slower than in the second period, is still rapid and the sugar concentration soon falls to the convulsive level. The other curves show intermediate characteristics.

The percentage drop in blood sugar was compared with the time of onset of convulsions. Ninety-four observations, on non-convulsive rabbits, gave a 30 per cent drop in blood sugar one-half hour after the injection of two units of insulin per kilogram of body weight. One hundred and sixty-one observations, on convulsive rabbits, were arranged into 3 groups depending on their percentage drop in blood sugar at the one-half hour interval. The following results were obtained:

GROUP	NUMBER OF OBSERVATIONS	AVERAGE PERCENTAGE DROP IN BLOOD SUGAR AT THE HALF HOUR INTERVAL	AVERAGE TIME OF ONSET OF CONVULSIONS
			minutes
B	54	28	194
C	53	43	160
D	54	56	112

The percentage drop in blood sugar at the half hour interval in the non-convulsive rabbits is the same as that of group B. This late convulsive group is very difficult to separate from the non-convulsive rabbits, in fact, when a rabbit varies from the non-convulsive group to a convulsive one in its response to weekly injections of insulin, it usually shifts to one or the other of these two groups. Figure 1 shows how similar these two groups are in their hyperglucemic responses to insulin. If a rabbit has a carbohydrate reserve capable of keeping it in either of these two groups, any slight change in this reserve can shift the animal from one to the other.

The above results indicate that the percentage drop in blood sugar, one-half hour after the injection of a fixed dose of insulin, is related to the time of onset of insulin convulsions.

An attempt was made to correlate the initial blood sugar concentration with the time of the onset of convulsions. The rabbits were divided into four groups, according to their response to insulin, in the same manner as was described on page 540 and gave the following results.

NUMBER OF OBSERVATIONS	GROUP	AVERAGE TIME OF ONSET OF CONVULSIONS	INITIAL BLOOD SUGAR, MGM. PER 100 CC.	ϵ	ϵ_M
		minutes			
95	A	No convulsions	106	11.4	1.2
51	B	237	103	12.0	1.7
74	C	139	102	9.8	1.1
37	D	80	106	8.9	1.5

From these results, it is apparent that the time of onset of convulsions is in no way related to the initial blood sugar value. This is in agreement with the results of Scott and Dotti (10). They found that the relative change in blood sugar, following insulin injection, is independent of the initial blood sugar value.

It will be noted that the initial blood sugar concentration, for these groups of rabbits, varies from 102 to 106 mgm. per 100 cc. of blood. This value is lower than that of 120 mgm. obtained by Dotti (11), with the original Shaffer and Hartmann method. This difference led us to compare the two methods, and it was found to be due to the difference in sensitivity of the two reagents to the non-fermentable reducing substances in the blood.

The reducing power of the blood, during convulsions, was found to be 28 mgm. per 100 cc. of blood in 129 observations. The mean deviation was 4.4 and the mean deviation of the mean was 0.4. This value is lower by 10 mgm. than the 38 mgm. reported for the rabbit by Dotti (11). This difference also is due to the greater sensitivity of the earlier method to the non-fermentable reducing substances present. As reported in the previous paper (11), there is presumably no true sugar present at the onset of convulsions.

The carbohydrate reserve appears to be at least one important factor in determining the sensitivy of the animal to insulin. Ionesco, Cosmulesco and Tomesco (12) in a series of rabbits, on a high carbohydrate diet, obtained an initial hyperglucemia following the injection of 5 units of insulin per kilogram of body weight. They obtained no initial hyperglucemia in two diabetic patients following insulin injection, but after a week of insulin treatment, the diabetics gave an initial hyperglucemic response to insulin. They interpret these results to mean that the initial hyperglucemia is related to the nutritional condition of the subject, hence to the glycogen reserve at the animal's disposal. Burger (13) concludes that the initial insulin hyperglucemia depends on the glycogen reserve of the liver. Bodansky (14) found that the initial effect of severe liver damage is a hyperglucemia followed by a hypoglucemia. Sprague (15) found that Eck fistula dogs, which have one type of chronic hepatic damage or insufficiency, are more sensitive to insulin than normal dogs.

From the results of the above authors (12) (13) the initial insulin hyperglucemia is related to the glycogen reserve at the animal's disposal. The amount of hyperglucemia can be used as an index of the amount of glycogen stored in the liver or perhaps in the whole body. If the initial insulin hyperglucemia is related to the glycogen reserve of the animal, and the sensitivity of the animal to insulin depends on its glycogen reserves, it follows that the greater the initial insulin hyperglucemia, the greater will be the resistance of the animal to insulin.

On this hypothesis it may provisionally be assumed that following insulin injection, the increased "demand" of the tissues for sugar causes a fall in blood sugar. This fall in blood sugar causes an increase in the rate of mobilization of glycogen, possibly through an increase in the adrenaline discharge. The ability of the rabbit to respond will depend on the glycogen reserve of the liver. The rabbits in group A, which gave the greatest initial insulin hyperglucemia and therefore must have the greatest carbohydrate reserve, will be able to respond to the hypoglucemia and supply enough glucose to prevent the total disappearance of glucose from the blood until the excess insulin in the blood is removed either by destruction or excretion. These rabbits, therefore, do not go into insulin convulsions. The rabbits in this group show, 6 hours after the insulin injection, a very definite trend of the blood sugar towards the normal. The rabbits in group B, which did not give as great an initial response to insulin as group A, were only able to supply the necessary amount of sugar to the blood for about 3 hours, and they then went into convulsions. The rabbits of group C, which gave the smallest initial hyperglucemia in response to insulin, were only able to meet the demand of the blood for carbohydrate for a shorter interval of time than either groups A or B and consequently went into convulsions sooner. The rabbits in group D, which gave no initial insulin hyperglucemia indicating a very poor carbohydrate reserve, went into convulsions in the shortest time interval.

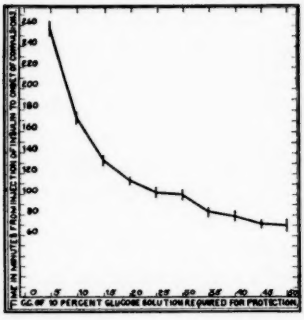

Fig. 5. Relationship between the time of onset of convulsions and the amount of glucose required for the prevention of subsequent convulsions. The vertical lines represent the precision with which the several points were established. The length of the lines represents ϵ_M.

The following observations are in agreement with the statement that the sensitivity of a rabbit to insulin depends on its glycogen reserve. The rabbits were injected with 5 cc. portions of a 10 per cent glucose solution at the onset of each convulsion.

Figure 5 shows the relationship between the amount of glucose, on this basis, that it was necessary to inject and the time of onset of convulsions in minutes. The graph shows that the greater the sensitivity of a rabbit to insulin, as measured by the time of onset of convulsions, the greater the amount of glucose which must be injected to prevent subsequent convulsions. As the animal's ability to supply carbohydrate to the blood in response to hypoglucemia produced by insulin depends on its carbohydrate reserve, the lower the carbohydrate reserve of the animal, the sooner the animal will go into convulsions, and the greater the amount of glucose which must be injected to balance the excess of insulin, so as to prevent subsequent convulsions.

There was no relationship found between the weight of the rabbit and the time of onset of convulsions, when the rabbits were injected with 2 units of insulin per kilogram. This is in agreement with the conclusion of Scott and Dotti (10) that when the dose is measured in units per kilogram of body weight, the effects of the insulin are strictly comparable, regardless of the body weight.

The response of a rabbit to an initial injection of insulin seems to be related to the sex of the animal. The males showed a decided resistance to insulin as compared with the females. The following observations show this. Sixty-two males received an initial injection of two units of insulin per kilogram of body weight. Twenty-eight went into convulsions within six hours after the insulin injection. That is 45 per cent suffered convulsions. Sixty-eight females were similarly injected and 53 went into convulsions. That is, 78 per cent suffered convulsions.

Sahyun and Blatherwick have reported that white or partly white rabbits are exceptionally resistant to insulin (16). We have not been able to associate any differences in response with the color of the subject. When 10 white females were given their initial injection of two units of insulin per kilogram, 70 per cent suffered convulsions. This is to be compared with the 77 per cent of 44 chinchilla females which were similarly injected. Both of these groups compare well with the 78 per cent shown by 68 females of mixed color. Of 19 white males, 47 per cent suffered convulsions, while 28 chinchilla males showed a similar response of 43 per cent. These values agree well with the 45 per cent shown by 62 males of mixed color.

The female rabbits show a greater development of sensitivity to insulin than the males when subjected to weekly injections. In order to study these mass changes in sensitivity to insulin, the rabbits were injected weekly with 2 units of insulin per kilogram of body weight. The response to the initial dose was recorded and they were further "sensitized" by two additional weekly injections. The responses to all injections following the third were then averaged and compared with the initial response

with the following results. Fourteen males, which suffered convulsions after the initial injection of insulin, gave an average convulsion time of 204 minutes. The average time after the third injection was 186 minutes. Nineteen female rabbits, which suffered convulsions after the initial injection of insulin, gave an average convulsion time of 194 minutes. The average time after the third injection was 141 minutes. Nine males did not suffer convulsions following the initial injection of insulin. After the third injection these nine males suffered convulsions following 51 per cent of the injections. The average convulsion time for these animals after the third injection was 212 minutes. Seven female rabbits did not suffer convulsions following the initial injection. After the third injection they suffered convulsions following 69 per cent of the injections. The average convulsion time for these animals after the third injection was 169 minutes. Seven rabbits, which did not go into convulsions at any time, consisted of six males and one female. Table 1 shows the variation in the response of different individuals to a weekly injection of two units of insulin per kilogram.

All the observations on 52 females were collected together and gave the following results. In 534 observations, there occurred 402 convulsions, and the average time of convulsions was 134 minutes after the injection. The mean deviation was 70 and the mean deviation of the mean was 3.4. In 543 observations on 44 males, there were 257 convulsions, and the average time of convulsions was 191 minutes after the insulin injection. The mean deviation was 92 and the mean deviation of the mean was 5.7.

The above observations indicate that the female is more sensitive to insulin than the male, both in its initial response to insulin injection, and in its subsequent responses to repeated insulin injection. The female also shows a greater consistency in its response to insulin than the male.

The weekly injection of 2 units of insulin per kilogram of body weight was associated with a slight increase in the body weights of the males and a small decrease in the body weights of the females. Twenty-five males, with an average initial weight of 2.43 kgm., showed an average weight of 2.59 kgm. after the third week of injection. Twenty-seven females, with an average initial weight of 2.76 kgm., gave an average weight of 2.56 kgm. after the third week of injection.

The continual injection of 2 units of insulin per kilogram of body weight seems to lower the resistance of the animal to infection. These rabbits showed a greater number of deaths due to sniffles and pneumonia than did control rabbits kept in the animal room.

The usual immediate cause of death during insulin convulsions is apparently respiratory failure caused by spasm of the muscles of respira-

TABLE 1

The response of the individual to weekly injections of two units of insulin per kilogram of body weight

RABBIT NUMBER	SEX	NUMBER OF OBSERVATIONS	NUMBER OF CONVULSIONS	PER CENT OF CONVULSIONS	AVERAGE TIME OF ONSET OF CONVULSIONS	ϵ	ϵ_M
					min.		
1	Female	35	34	97	77	24	4
3	Female	8	8	100	82	5	2
9	Female	53	45	85	85	35	5
10	Female	38	38	100	87	21	3
45	Female	10	10	100	94	35	11
6	Female	21	20	95	96	40	9
12	Female	9	9	100	98	27	9
52	Female	16	16	100	108	27	7
11	Female	10	8	80	117	36	13
54	Female	8	7	88	120	39	15
4	Female	22	19	86	132	91	29
2	Female	17	15	88	159	93	24
77	Female	16	9	56	169	64	21
7	Female	35	34	94	170	63	11
90	Female	11	6	55	177	65	23
89	Female	14	12	86	178	73	21
93	Female	13	5	38	180	102	46
13	Female	25	19	76	183	63	14
91	Female	15	6	40	183	96	38
94	Female	14	10	71	184	45	14
79	Female	13	7	54	189	106	41
28	Female	21	9	43	191	60	20
88	Female	14	8	57	230	49	17
74	Female	13	5	38	254	24	11
30	Female	10	0	0			
44	Male	24	24	100	77	25	5
58	Male	7	7	100	88	25	10
57	Male	10	9	90	109	35	12
8	Male	22	21	95	119	46	10
5	Male	23	11	47	142	42	19
92	Male	14	3	21	148	80	47
51	Male	37	29	78	150	60	11
55	Male	12	9	75	164	18	6
56	Male	12	5	42	165	52	24
82	Male	12	10	83	171	55	18
53	Male	8	8	100	183	66	24
36	Male	14	5	36	187	65	30
76	Male	14	5	36	226	86	39
15	Male	37	31	84	240	59	10
20	Male	13	10	77	264	58	18
14	Male	9	9	100	271	53	18
63	Male	25	15	60	292	83	21
67	Male	24	6	25	304	41	17
96	Male	12	4	33	324	28	14
18	Male	38	0	0			
19	Male	38	0	0			
64	Male	9	0	0			
75	Male	12	0	0			
78	Male	12	0	0			
95	Male	12	0	0			

tion. The injection of glucose does not always remedy this condition. It was found that death could be prevented if, following the glucose injection, the animal were given artificial respiration.

The author desires to express his sincere thanks and appreciation to Dr. E. L. Scott for his encouragement, guidance and criticism given throughout this investigation. I wish to thank Dr. H. B. Williams and Dr. A. H. Scott for their advice and criticism in the preparation of the manuscript.

SUMMARY

1. Insulin hyperglucemia offers a means of determining the sensitivity of an animal to insulin, without subjecting it to insulin convulsions.

2. The percentage drop in blood sugar, one-half hour after the injection of a fixed dose of insulin, is related to the time of onset of convulsions.

3. The time of onset of convulsions, following insulin injection, is in no way related to the initial blood sugar concentration.

4. The greater the sensitivity of a rabbit to insulin, as measured by the time of onset of convulsions, the greater the amount of glucose which must be injected to prevent subsequent convulsions.

5. The female rabbit is more sensitive to insulin than the male, both in its initial response to insulin injection and in its subsequent responses to repeated insulin injections. The female is more constant in its responses to insulin injections than the male.

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THE BLOOD SUGAR LEVEL AFTER ADMINISTRATION OF PILOCARPINE, ATROPINE AND ACETYL CHOLINE

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It is generally recognized that the mechanism by which the concentration of the sugar in the blood is controlled is very sensitive and very effective. It is also generally conceded that the endocrine function of the pancreas is one of the important factors in this control and that epinephrine is another. The relations of the epinephrine function of the adrenals to the autonomic nervous system have been fairly well established, but the mechanism by which insulin discharge is coördinated with the needs of the organism is still largely a matter of speculation.

The present paper is the report of a research which it was hoped would throw some light upon the nervous factors involved in the control of the concentration of the sugar in the blood.

The method of approach is through specific autonomic stimulation by drugs. For this purpose three drugs have, so far, been studied.

1. Pilocarpine, usually thought to stimulate the parasympathetic nervous system.
2. Atropine which paralyzes this system.
3. Acetyl choline which is released at the cholinergic effector during parasympathetic stimulation.

If the vagus nerves are excitatory to the Islets of Langerhans, then on the basis of classical theory, the injection of pilocarpine would cause a liberation of insulin, with a resulting hypoglycemia, and atropine, according to its usual effect, would cause a rise in the blood sugar. The evidence, however, is conflicting for, although Macleod and Ruh (1) working on dogs, reported that atropine in doses of 1 mgm. per kilo produced a hyperglycemia and although MacGuigan (2) also found on dogs a hyperglycemia with large doses of atropine, other workers have found quite the reverse. Lang (3) reported results of 27 experiments on rabbits. After the injection of atropine up to 0.5 mgm. per kilo, there was a decrease in the blood sugar ranging from 8.8 to 24.7 per cent. MacGuigan (2) found that pilocarpine never caused a rise in the blood sugar of dogs but often a fall for some hours. Nitzescu and Benetato (4) found an

increase of over 300 per cent in the blood sugar of dogs injected with 5 mgm. of pilocarpine per kilo.

Dale and Laidlaw (5) suggested that the action of pilocarpine on uterine muscle simulated that of sympathetic nerve stimulation. Atropine immediately abolished the pilocarpine effect. They reported that the output of epinephrine was at least doubled by pilocarpine injection. They suggested that pilocarpine stimulates the sympathetic ganglion cells. Stewart and Rogoff (6) found no material change in the rate of output of epinephrine from the adrenals in cats after the injection of either atropine or pilocarpine.

The lack of uniformity in the experimental results of the different workers with these drugs makes conclusions impossible and justifies further work on the subject.

It is now generally accepted that the parasympathetic nervous system acts through the liberation of acetyl choline. This drug offers, therefore, the closest approximation to a physiological stimulation. In 1906 Ried Hunt (7), working with the blood pressure-reducing principle of suprarenal and brain, ascribed the fall in blood pressure to acetyl choline. He stated that he was "inclined to think it is due to an effect upon the terminations of the vagus in the heart." In 1934, Dale (8) reported, "it can now be definitely stated that the vagus impulses produce their effects by liberating acetyl choline . . . among the fibers of the muscular wall of the heart."

All of our experiments were performed upon intact normal-fed rats. The effects, therefore, presuming that the drugs do act through the channels indicated, measure the integrated effect of sympathetic or parasympathetic stimulation upon the whole mechanism by which the concentration of the sugar in the blood is controlled. The effect upon specific organs cannot be isolated by these means.

Pilocarpine. Pilocarpine HCL was used in doses of 5 mgm. per kilo. The accompanying chart, figure 1, curve *1*, shows a significant rise in the blood sugar level in 15 minutes with a return to the original level in one hour.

Atropine. Atropine sulphate was used in doses of 20 mgm. per kilo. Figure 1, curve *2*, shows a significant fall in the blood sugar level in 1½ hours with a return to the original level in 2 hours. In view of the fact that MacGuigan reported large doses of atropine to produce a rise in the blood sugar level, we tried several small test series with doses ranging as high as 65 mgm. per kilo. In no case did we obtain a rise.

Pilocarpine after atropine. It will be noted that there is no demonstrable effect of atropine during the first half-hour. On the basis of this, another series of rats was injected with atropine and at the half hour, again injected with pilocarpine in the dosages already indicated. Figure 2, curve *3*, shows that no pilocarpine effect resulted after the atropine.

Acetyl choline. Acetyl choline HBR was used in a concentration of 1:25 molar. The dosage employed was 1 cc. per kilo. As can be seen from figure 2, curve *4*, there is a sharp fall in the blood sugar level, the greatest change occurring at the 20-minute interval. The blood sugar level has not returned to the original level in 2½ hours.

Conclusion. The surprising disagreement between acetyl choline and pilocarpine in their effect upon the blood sugar level renders a definite conclusion based upon their action as to the innervation of the islets premature. The following is offered as a suggested explanation of the effects. Pilocarpine does raise the blood sugar level and atropine lowers it. Atropine also prevents the rise following pilocarpine. If, in the face of classical interpretation, one can accept the postulates of Dale that pilocarpine stimulates the sympathetic ganglion cells and that of Cannon and Cattell (9) that atropine paralyzes the medullary cells of the adrenals, these results may be explained by assuming a disbalance of

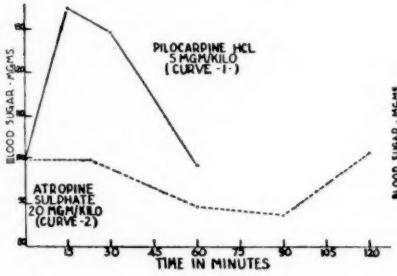

Fig. 1

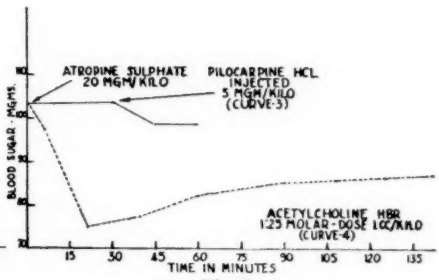

Fig. 2

Figs. 1 and 2. Fifty observations in each point of each curve

insulin and epinephrine, with a normal supply of insulin in either case. Thus, following pilocarpine, there is an excess of epinephrine with normal insulin and with atropine, an epinephrine insufficiency with again a normal insulin. It is also possible that these drugs do have their usual parasympathetic effects which, in turn, may be masked by the direct action upon the adrenal medulla.

With acetyl choline, on the other hand, there is apparently a direct vagal effect, motivating islet discharge with a consequent hypoglycemia resulting from the excess insulin.

SUMMARY

1. Pilocarpine raises the blood sugar level. The maximum effect is produced in fifteen minutes.

2. Atropine lowers the blood sugar level. The maximum effect occurs in ninety minutes.

3. Atropine prevents the rise after pilocarpine.

4. Acetyl choline lowers the blood sugar level. The lowest point is reached in twenty minutes.

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THE EFFECT OF EXPERIMENTAL HYPOTHALAMIC LESIONS UPON BLOOD SUGAR

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Although systematic studies of the participation of the hypothalamus in carbohydrate metabolism have been relatively few, numerous observations have been recorded which seem to indicate its possible significance in this respect. Considerable disagreement and confusion exist, however, especially in regard to localization of function. Hyperglycemia and glycosuria have been observed to follow injury to the hypothalamus in experimental animals by Aschner (1), Sachs and MacDonald (2), Camus, Gournay and LeGrand (3), Lewy and Gassmann (4) and others. Such elevations of the blood sugar have in most instances been transient. Hyperglycemias produced as a result of direct electrical stimulation of the hypothalamus have been reported by Himwich and Keller (5), Magoun, Barris and Ranson (6), and Miki (7). Conversely, low blood sugars following the production of lesions in the chiasmal region have been observed by Miki (7) and D'Amour and Keller (8). All authors are not agreed, however. DeWulf (9) was unable to confirm the work of Camus, Gournay and LeGrand (3); Houssay (10) denies that hypoglycemia is associated with lesions in the hypothalamus. Macleod (11) is dubious as to any relationship between the hypothalamus and carbohydrate metabolism, although he has observed hyperglycemia in rabbits after decerebration at the pontine level.

Attempts at localization of glycoregulatory function in the hypothalamus chiefly rest upon the work of Camus, Gournay and LeGrand, who state that injury to the paraventricular nucleus produced glycosuria in rabbits, and of Miki who found hypoglycemia after injury to this structure.

The present authors have accumulated considerable data as to the behavior of the blood sugar in an extensive series of cats in which lesions affecting various parts of the hypothalamus have been produced. A summary of these results is herewith presented.

METHODS. The lesions were produced electrolytically with a fine unipolar electrode oriented by the Horsley-Clarke stereotaxic instrument, the operations being performed under aseptic precautions. The cats,

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before and after operation, received a measured diet of ground beef heart and milk. Certain of them, which became cataleptic for a time after lesions had been placed in the posterior part of the hypothalamus, refused food and were fed by tube. A period of 18 to 24 hours was allowed to elapse between feeding and bleeding. Blood samples were taken from the saphenous vein. The Randles-Grigg (12) modification of the Folin-Wu technique was used for estimation of the blood sugar, 0.5 cc. samples being utilized. Only the results from animals in which the blood sugar picture was uncomplicated by infection are reported. Cats for which the number of blood sugar determinations was considered inadequate have also been excluded.

At the conclusion of the period of observation the cats were killed by bleeding, the heads injected with formalin (U.S.P., 1:10) and the brains removed. The diencephalic regions were embedded in celloidin, cut into 50μ sections, every third and fourth of which was stained alternately with cresyl violet and Weil's stain, and mounted. The anatomical terminology used is that of Ingram, Hannett and Ranson (13). This applies especially to the nucleus designated by some authors as the nucleus paraventricularis, which is here called the nucleus filiformis.

Observations. In the majority of cases blood sugar estimations were made before operation. Blood sugar determinations have been made on 90 normal cats, a total of 108 samples, since 24 determinations were made at different times on a group of 6 of these animals. The mean result of these 108 estimations was 86.1 mgm. of glucose per 100 cc. of blood. The mean of 24 estimations on 6 cats was 80.2 mgm. per cent. Taking the standard deviation into consideration, however, the normal blood sugar range of cats has been considered to be 75 to 100 mgm. per cent.

The effects of bilateral hypothalamic lesions upon the blood sugar have been observed in 55 cats. The number of blood sugar determinations for each animal has varied from 2 to 24, depending upon the length of the survival period, which in some cases has reached 290 days. The general location of the lesions has been considered by arbitrarily dividing the hypothalamus into three zones, as follows. The anterior hypothalamic zone lies dorsal to the chiasm and includes the caudal part of the preoptic area. The tuber zone lies between the chiasm and the premammillary area and includes the infundibulum. The posterior zone includes the premammillary, mammillary and supramammillary areas as well as the posterior hypothalamic nucleus. It must be understood that the lesions were not always confined to a single one of these zones, but frequently extended over parts of two, or even three of them.

Hyperglycemia. Forty-two animals showed hyperglycemia following operation. Most of the blood sugars in the immediately post-operative period were above 150 mgm. per cent, the range being 122 to 416 mgm.

The period of hyperglycemia ranged from 1 to 22 days, an average of 3.7, but this condition was apparently transient, since those cats with a sufficiently long survival period returned to normal or subnormal levels. Sixteen cats failed to survive the period of hyperglycemia, the chief cause of death being supervening infection. It was found in a number of cases that insulin was effective in reducing the blood sugar level. Study of the respiratory metabolism of one animal during the hyperglycemic phase revealed a diabetic respiratory quotient (R.Q. 0.657, with blood sugar 267 mgm. per cent), and glycosuria and ketonuria were noted.

According to the anatomical findings in this group, the anterior hypothalamus was injured in 20 cats, the tuber region in 15 cats and the posterior hypothalamus in 17. There was injury or atrophy of the nucleus filiformis in 22 animals.

Hypoglycemia. Definite tendency to run low blood sugars at times was found in 10 cats. This tendency was usually noted at a considerable interval after operation and persisted for some time—in most instances throughout the life time of the animal. The word "persist," however, is perhaps misleading, for in 7 of these cases the low blood sugars were intermittent, figures in the low normal range occasionally appearing. The lower range of blood sugars varied from 46 to 62 mgm. per cent. The animals of this group were kept under observation for periods of from 23 to 228 days. Four of these cats which showed hypoglycemic tendencies for especially long periods have been made subjects of special experiments which have produced evidence that actual disturbance of carbohydrate metabolism may occur in these animals.

The anatomical findings on the 10 cats of this group with hypothalamic lesions show that the anterior hypothalamus was affected in 8 cases, the tuber region in 2 and the posterior hypothalamus in 2. There was injury or atrophy of the nucleus filiformis in 8 instances.

Normal blood sugars. Seven cats with hypothalamic lesions and 3 cats with lesions in the cerebral hemispheres or dorsal thalamus ran normal blood sugars throughout the post-operative period, the transient hyperglycemic phase being absent. Of the animals with hypothalamic damage, the anterior hypothalamus was involved in 3, and the tuber region in 6. The posterior zone was not affected. There was injury or atrophy of the nucleus filiformis in 4 instances.

COMMENT. It has seemed permissible to attempt correlation of the various blood sugar findings with the injury to the different portions of the hypothalamus, as follows. Attention should be called to the fact that an animal showing hyperglycemia for a time may later run a normal or low blood sugar. Thus it may be considered below as both transient hyperglycemia and hypoglycemia, or as transient hyperglycemia and eventually normal. It should not be forgotten that the hyperglycemia is a transient,

acute phenomenon, and that the later condition of the animal must be taken into consideration. It is quite possible also that these cats which succumbed during the hyperglycemic period might be considered as potentially normal. Also, damage to one part of the hypothalamus may extend into another part, and an animal may be listed twice for this reason. This accounts for apparent discrepancies in the totalizing of percentages given below.

Lesions have been placed in the anterior hypothalamic zone in 27 cats. The blood sugar distribution is as follows:

Transient hyperglycemia	19, or 70.3 per cent
Hypoglycemia	8, or 29.6 per cent
Blood sugars normal throughout	4, or 14.8 per cent
Normal plus eventually normal	8, or 29.6 per cent

Lesions in the tuber region were found in 23 cats. The blood sugar distribution, applying the criteria assumed in the preceding paragraph, follows:

Transient hyperglycemia	16, or 69.5 per cent
Hypoglycemia	2, or 8.7 per cent
Normal throughout	6, or 26.0 per cent
Normal plus eventually normal	15, or 65.2 per cent

There were 18 cats with lesions in the posterior hypothalamic zone.

Transient hyperglycemia	17, or 94.4 per cent
Hypoglycemia	2, or 11.1 per cent
Normal throughout	0
Eventually normal	11, or 61.1 per cent

There is little difference in the relative degree of hyperglycemia produced by lesions in the three zones, the average peak figures being 180 mgm., 167 mgm. and 170 mgm., respectively.

It would seem from this angle that the greatest incidence of hyperglycemia occurs after lesions in the posterior portion of the hypothalamus, the anterior and tuberal regions also having a high proportion. As to low blood sugars, however, the anterior zone is far ahead of the others. The percentage of consistently normal blood sugars is greatest with lesions in the tuberal region, but in the matter of eventually normal figures the tuber and posterior hypothalamus both outstrip the anterior area, 65 and 61 per cent for the former to 30 per cent for the latter.

The condition of the nucleus filiformis has been carefully studied in the whole series of cats in order to determine the degree of destruction or atrophy undergone in each instance. Atrophy of this structure has been observed, especially when the lesions were placed in the suprachiasmatic region in the neighborhood of the fornix and the area immediately ventral to the latter, presumably interrupting an efferent pathway. Such lesions

frequently also produce atrophy of the nucleus supraopticus and of the posterior hypophysis, conditions associated in our experience with diabetes insipidus in the cat. No definite atrophy of any part of the hypophysis has been found following destruction or atrophy of the nucleus filiformis, provided the supraoptico-hypophyseal tract was not injured.

The relation of the state of the filiform nucleus to the blood sugar findings may be presented as follows:

1. Complete destruction or marked atrophy of the nucleus filiformis—7 cats.
   - Transient hyperglycemia ..... 3, or 42.8 per cent
   - Hypoglycemia ..... 4, or 57.0 per cent
   - Normal throughout ..... 2, or 28.5 per cent
   - Normal and potentially normal ..... 3, or 42.8 per cent
2. Nucleus filiformis partially destroyed or atrophic—23 cats.
   - Transient hyperglycemia ..... 19, or 82.6 per cent
   - Hypoglycemia ..... 4, or 17.3 per cent
   - Normal throughout ..... 2, or 8.2 per cent
   - Normal and eventually or potentially normal ..... 19, or 82.6 per cent
3. Nucleus filiformis unaffected—21 cats.
   - Transient hyperglycemia ..... 16, or 76.2 per cent
   - Hypoglycemia ..... 3, or 14.3 per cent
   - Normal throughout ..... 3, or 14.3 per cent
   - Normal and eventually normal ..... 17, or 80.9 per cent

If comparisons are valid in this instance it would seem that cats with normal or only partially destroyed filiform nuclei tend to show a greater proportion of transient high and eventually normal blood sugars and a lower percentage of low blood sugars than do animals with complete destruction or atrophy of this structure. There may be some question as to the acceptability of a comparison involving so few as 7 animals on the one hand and as many as 23 on the other, but in the authors' opinion there is a greater tendency toward the occurrence of low blood sugar concentrations in cats with lesions in the anterior hypothalamus than in cases in which the lesions are located elsewhere, the results tending to support Miki (7) and D'Amour and Keller (13). Furthermore, low blood sugars associated with lesions in the more posterior portions might possibly be accounted for by the involvement of descending pathways from the anterior zone. On the other hand, there appears to be a lower incidence of transient hyperglycemia among animals with complete destruction of the nucleus filiformis than in those with partial destruction or no direct involvement of the nucleus, tending to support the findings of Camus, Gournay and LeGrand.

The actual direct reasons for the occurrence of hyper- and hypoglycemias are obscure. In view of the evanescence of the hyperglycemic phase the most logical explanation seems to lie in irritation of neurons or fibers. In cases where the lesion closely approaches the pituitary fossa one might be

tempted to consider irritation of the anterior hypophysis. However, in a great number of our cases the hypophyseal region was not approached. As to the possible effector mechanisms by which the hyperglycemia may be produced the reader is referred to the papers of Macleod (11, 14, 15). If the high blood sugars are due to irritative processes, it would seem that the low blood sugars might be due to a deficiency of some sort. The mechanism for this is as yet unknown.

It should be pointed out that while lesions in the anterior portion of the hypothalamus and, more particularly, lesions of the nucleus filiformis are associated with the majority of cases of low blood sugars in the cats of this series, this finding is not absolute for individual cases. Normal and rather constant blood sugar levels have been observed in animals with extensive lesions in this region. One must be cautious about assigning a normal glycoregulatory mechanism to such a cat, for an animal with normal blood sugar may prove to be decidedly deficient when judged by other criteria. The results of other experiments along these lines are to be communicated shortly.

SUMMARY

The blood sugars were followed in 55 cats after production of lesions in various parts of the hypothalamus. Forty-two of these showed a post-operative hyperglycemia which was always transient and, provided the animal survived, returned to normal or subnormal levels. Seven cats showed no abnormality in the blood sugar level at any time after operation. Ten showed a tendency to mild, frequently intermittent, chronic hypoglycemia—some of these showed transient hyperglycemia in the early post-operative period. Analysis indicates that so far as groups of animals are concerned, transient hyperglycemia may occur after the production of lesions at almost any point in the hypothalamus. On the other hand, hypoglycemias are most frequent when the lesions are in the anterior portion of the hypothalamus, and in the majority of these cases the nucleus filiformis is destroyed or atrophic. There may be individual exceptions, however.

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EVIDENCE OF ALTERED CARBOHYDRATE METABOLISM IN CATS WITH HYPOTHALAMIC LESIONS

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A series of observations on cats with lesions in various parts of the hypothalamus (1) has shown that in certain of these animals there is a tendency to recurring low blood sugar concentrations. These on the whole tended to occur most frequently when there was injury to the anterior part of the hypothalamus, and more particularly when the nucleus filiformis was destroyed or atrophic. However, the effects of hypothalamic lesions on the blood sugar cannot be said to be uniform—some animals with injury to the anterior hypothalamic region never showed low blood sugars, and in at least one case of complete destruction of the nucleus filiformis the blood sugar usually remained at a normal level. It was, therefore, thought desirable to study the carbohydrate metabolism of such cats from other angles to determine if possible if a real derangement existed. Five of a series of cats with lesions in the anterior portion of the hypothalamus, dorsal to the chiasm and involving the filiform nuclei, were kept for some 8 months after operation. Four of these (cats 1, 2, 3 and 4) showed lowered blood sugars repeatedly, the other (cat 5) ran a rather consistent normal, with relatively rare low figures. These animals have been studied from the following aspects: *a*, the response of the blood sugar to small doses of insulin; *b*, the response of the blood sugar to epinephrine; *c*, the effect of repeated administrations of a saline extract of the anterior lobe of the hypophysis upon the blood sugar.

Additional experiments along certain of these lines have been carried out upon a group of 9 other cats with lesions in the anterior hypothalamic zone, and on 3 with practically complete removal of the hypophysis.

METHODS. Healthy adult cats of average size and equable disposition were used.

The hypothalamic lesions were produced electrolytically with the aid of the Horsley-Clarke stereotaxic instrument, the use of which has been described elsewhere.

The cats were maintained on a measured diet of ground beef heart and

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milk which was somewhat in excess of their actual needs. The quantity of food given was sometimes varied for purposes of experiment.

All blood samples were taken from the saphenous vein and the blood sugar concentration estimated according to the Randles-Grigg (2) modification of the Folin-Wu method, 0.5 cc. samples being used. The animals were fasted 18 to 22 hours before bleeding or other experimentation.

Sensitivity to insulin was studied according to the following method. Since the normal controls and experimental animals were of rather uniform size and weight (about 3 kgm.) and of uniform variation in the two groups, doses of 0.5 unit of insulin were given intravenously in each instance in order to avoid possible error in attempting finer dilution of the commercial insulin solution. The animal under observation was bled and the insulin injected immediately. Other blood samples were taken 30, 60, 120 and 180 minutes after injection.

Observations upon the response of the blood sugar to epinephrine were carried out in a similar manner, except that the epinephrine was administered subcutaneously in doses of 0.08 mgm. per kilo.

A saline suspension of anterior lobe of the beef hypophysis which was effective in elevating the blood sugar level in normal cats was prepared according to the method of Schockaert (3). Fresh preparations were made every 2 or 3 days and kept in the ice box. The glands were kept frozen until the time they were used in making the suspension.[2] In one group of experiments a quantity of this substance equivalent to 3 grams of fresh anterior lobe tissue was administered daily to each animal by the intraperitoneal route. In another series of experiments the dosage of anterior lobe was reduced to the equivalent of 1.5 grams each day.

The glycogen content of the liver was estimated in most instances at the end of the period of observation. This was carried out according to the method of Good, Kramer and Somogyi (4). The liver samples were removed immediately following anesthetization with sodium amytal given intravenously.

Hypophysectomy was attempted with at least partial success in 3 cats, using the retropharyngeal route of McPhail (5).

OBSERVATIONS. *Sensitivity to insulin.* Cats 1, 2, 3, 4, and 5, in which the lesions were placed in the medial portion of the anterior hypothalamic and preoptic areas, showed definitely increased sensitivity to insulin as compared to normal controls. The experiments were performed some 4 months after operation. A composite picture of the behavior of the blood sugar in these animals as compared with that of 5 normal controls is shown in figure 1. There was some variation in the degree of the response, the sensitivity being of the order $5 > 3 > 4 > 1 > 2$, and in the last two

[2] The authors wish to acknowledge the kindness of Dr. David Klein of the Wilson Laboratories in furnishing part of the material used.

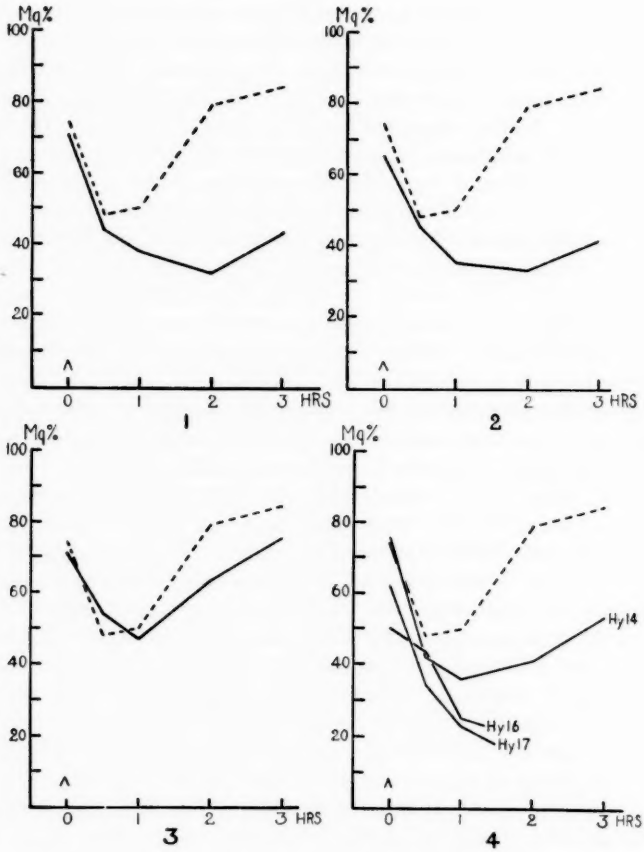

Fig. 1. Composite curves showing the sensitivity to insulin of cats 1, 2, 3, 4 and 5 (solid line) as contrasted with normal cats (broken line). ^ indicates time of insulin injection. Blood sugar in milligrams per 100 cc. blood.

Fig. 2. Composite curves showing the sensitivity to insulin of cats 8, 9, 10, 12 (solid line) as contrasted with normal cats (broken line).

Fig. 3. Composite curves showing approximately normal sensitivity to insulin in cats 6, 7, 13, 11, 14 (solid line) as compared with normal cats (broken line).

Fig. 4. Curves showing the sensitivity to insulin of cats Hy16, Hy17, with severe hypophyseal deficiency, and Hy14, with less hypophyseal deficiency. Normal curve is broken line.

named the increased sensitivity was manifest more in the prolongation of the period of blood sugar depression than in the depth of the latter. While the normal animals showed no noticeable physical symptoms whatever

during the experiment, the operated cats displayed some signs of shock, and in one animal (5) glucose administration became necessary, the blood sugar having fallen to 20 mgm. per cent or less.

Similar experiments were carried out on a group of 9 other cats. Two of these, cats 6 and 7, had unilateral lesions involving the anterior and lateral hypothalamic areas. Seven had bilateral lesions affecting the intermediolateral part of the anterior hypothalamic zone with caudal extensions involving the medial part of the tuber—these were designed to produce diabetes insipidus and 4 of the animals (cats 8, 9, 10 and 11) did have permanent polyuria.

Four of the 9 (cats 8, 9, 10 and 12) showed definitely increased sensitivity to insulin as illustrated by the composite graph in figure 2. In cat 9 the blood sugar dropped below 20 mgm. in 1½ hours and recovery did not occur. In cat 10 rather marked muscular incoördination developed, but in cat 8 and cat 12 the symptoms were relatively slight, and the chief abnormality of the blood sugar curve was its marked prolongation.

The other 5 cats (cats 6, 7, 13, 11, 14) showed practically normal responses to insulin, with perhaps a slightly delayed recovery of the normal blood sugar level, as indicated in the composite graph in figure 3.

In addition to the above, the insulin sensitivity was tested in 3 cats from which almost all of the hypophysis had been removed. Two of these (cats Hy16 and Hy17) showed very marked and uniform responses and glucose administration was necessary 1½ hours after injection of the insulin. The other showed a peculiar, prolonged curve. These results are illustrated in figure 4.

Response to epinephrine. Epinephrine, in doses of 0.08 mgm. per kilo, was given subcutaneously to cats 1, 2, 3, 4 and 5 and to 4 normal cats. Blood samples were taken just before injection and at hourly intervals thereafter for a period of 5 hours. While the blood sugar was elevated in the operated animals, reaching peak figures from 110 to 177 mgm. per cent, the elevation in no case approached that in the normal controls in which the highest figures were 194 to 237 mgm. In none of the animals did the blood sugar return to the normal range during the five-hour period. Composite curves showing the diminished response to epinephrine in the operated cats are given in figure 5.

Response to extract of anterior hypophysis. Intraperitoneal injection of the equivalent of 3.0 grams of fresh anterior lobe per day in 5 normal cats produced marked elevations of the blood sugar. The number of daily injections required varied somewhat and appeared to depend to considerable extent upon the quantity of food consumed. After 4 days of injection, the blood sugar rose to 165–182 mgm. per cent. Continued administration of the extract over a period of 10 days produced rises as high as 384 mgm. in some instances. A composite graph showing the course of the

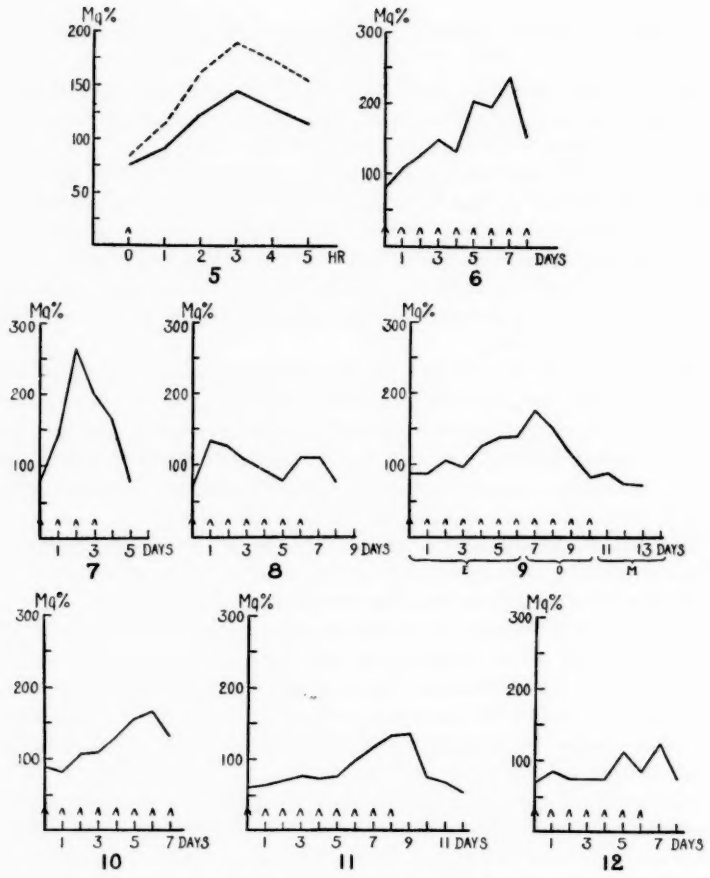

Fig. 5. Composite curves showing response of the blood sugar to epinephrine in cats 1, 2, 3, 4 and 5 (solid line) as compared with normal cats (broken line).

Fig. 6. Composite curve showing response of the blood sugar to daily injection of anterior lobe suspension in normal cats. ^ indicates injection of 3.0 grams anterior lobe.

Fig. 7. Composite curve showing the blood sugar response to daily injections of 3.0 grams anterior lobe in two normal cats on excess diet.

Fig. 8. Composite curve showing the blood sugar response to daily injections of 3.0 grams anterior lobe in cats 2, 3, 4 and 5 on maintenance diet.

Fig. 9. Composite curve showing the blood sugar response to daily injections of 3.0 grams anterior lobe in cats 3, 4, and 5 while on excess diet, fasting and maintenance diet. *E* indicates periods of excess diet, *O* indicates period of fasting, *M* indicates period of maintenance diet.

Fig. 10. Composite curve showing blood sugar response to daily injection of 1.5 grams anterior lobe in 4 normal cats.

Fig. 11. Composite curve showing blood sugar response to daily injections of 1.5 grams anterior lobe in hypophysectomized cats Hy14, Hy16 and Hy17.

Fig. 12. Curve showing blood sugar response to daily injections of 1.5 grams anterior lobe in cat 8.

blood sugar in the 5 normal cats over a period of 8 days is given in figure 6. Two normal cats were given an excess diet for 6 days and during this period 4 daily doses of 3.0 grams anterior lobe were given. The response was very prompt and on the third day of injection blood sugars of 241 and 283 mgm. were noted. This is illustrated in figure 7.

Cats 1, 2, 3, 4 and 5 were subjected to such treatment during two periods. In the first period injections were given for 7 days while the animals were on a maintenance diet. During the second period 4 of these animals received 7 daily injections while on an excess diet and, immediately after this, 4 injections while being starved. Cat 1 responded in a typically normal fashion while on the maintenance diet, the blood sugar rising rapidly to reach a peak of 454 mgm. by the sixth day. During the second period the rise was more gradual, reaching 277 mgm. on the sixth day. The other animals were definitely refractory to the effect of the extract. The elevations secured were of a relatively low order and arrived at their peak within a day or two after the initial injection—from there the blood sugars fell off and usually fluctuated in the upper normal range. Following cessation of the injections normal or subnormal levels were quickly reached. A composite curve showing the behavior of the blood sugar in these cats is shown in figure 8, for comparison with the normal curve in figure 6. During the second period, while on excess diet, a really high blood sugar was reached in only one of this group, the blood sugar rising in this cat to a peak of 306 mgm. on the seventh day. The composite curve for 3 of these animals (fig. 9) shows that during excess feeding the rate and degree of blood sugar elevation did not compare with that shown by the normal animals, even when the latter were on a normal or maintenance diet, while the rate of elevation of the 2 normal cats on excess diet was much more rapid than that of the operated animals.

Unfortunately for purposes of comparison, such large doses of anterior lobe were not given to cats with hypophyseal deficiency. However, experiments were tried in which 1.5 gram dosages of anterior lobe were used. The effect of these small quantities on the blood sugar of 4 normal cats on a somewhat variable excess diet is shown in the composite graph in figure 10. The response was rather gradual and peak figures of 180 to 225 mgm. were reached. In the 3 hypophysectomized cats some difficulty was experienced, and 7 to 9 daily injections were required to produce such rises as were observed. The maximum levels reached were 130, 148 and 155 mgm. and the delayed nature of the response is illustrated in figure 11. It may be that the compensation required for the general deficiency present was responsible for this tardiness.

Cat 8 was also treated with the 1.5 gram doses of anterior lobe. As figure 12 indicates, this animal showed a deficient response as compared with the normals. A peak of 125 mgm. per cent was reached after 7 days.

Glycogen content of the liver. Estimations of the liver glycogen of the various experimental animals were made in order to see if there were any indications that the abnormalities observed could be due to deficiency in this regard. While the determinations were made long after the experiments had been performed, the animals were being kept under the same conditions and were in the same state of general good health.

The liver glycogen content of 4 normal cats was found to be 3.1, 3.63, 5.65 and 4.23 per cent, an average of 4.15 per cent. These results agree with the findings of Sacks, who found that in 22 normal cats the glycogen content varied from 1.28 to 6.15 per cent, with an average of 3.29 per cent. In 9 operated animals, including 7 showing abnormal sensitivity to insulin and 2 displaying normal reaction, the liver glycogen was decidedly normal, the values ranging from 3.09 to 5.51 per cent.

In 2 hypophysectomized cats the liver glycogen content was normal—5.33 and 3.87 per cent. One, cat Hy17, however, ate practically no food for a period of 6 days before it was killed and under these circumstances the liver glycogen was 0.3 per cent. This does not mean, of course, that the glycogen content was not normal at the time the insulin experiments were performed, for at that time the cat was well nourished and consuming its portion of food regularly. It will be recalled that Corkill, Marks and White (7) and Cope and Marks (8) report that the liver glycogen of hypophysectomized rabbits is normal.

In two normal cats the liver glycogen was estimated the day after a series of seven 1.5 gram doses of anterior lobe had been given. The results were on the high side of normal—5.82 per cent and 6.64 per cent.

Location of the lesions. Examination of microscopic sections of the brains of the experimental animals was carefully carried out, but no attempt at detailed description of the anatomical findings will be made here.[3]

Viewed as a group, however, one receives the impression that the chief differences between the lesions in cats which were unusually sensitive to insulin and in those which reacted normally are:

Slightly greater extent of the lesions in the insulin-sensitive cases.

Greater involvement of the anterior hypothalamic region and ventricular wall in the insulin-sensitive cats.

Much greater injury or atrophy of the filiform nuclei in the insulin-sensitive animal. In all cats of this type the filiform nucleus was either completely or almost completely destroyed or markedly atrophic, while in the cats which reacted normally, damage to this structure was very slight or entirely lacking. It is necessary to mention, however, that cat 1, in which there was complete bilateral destruction of the nucleus filiformis,

[3] Typed descriptions of the lesions will be furnished to interested persons who desire to communicate with the authors.

reacted in a normal manner to anterior lobe injections, and while its response to insulin was abnormal it was not one of the striking cases.

No direct damage to the hypophysis. Except for those individuals showing permanent polyuria, in which the pars nervosa was exceedingly atrophic, no visible evidence of injury to the hypophysis was detectable in any case.

COMMENT. The above results have certain features in common with those described for animals under other experimental conditions. Thus, it is well known that similarly marked responses to insulin are found after hypophysectomy and partial or complete removal of the adrenals. The same is true after splanchnic section, sympathectomy, or high thoracic spinal cord section (Rupp, 9; Dworkin, 10; Schlossberg, Sawyer and Bixby, 11; Brooks, 12), and after subtemporal exposure of the hypophysis without removal of the latter (Chaikoff, Reichert, Larson and Mathes, 13). Decreased responses to epinephrine have also been observed following hypophysectomy (Cope and Marks, 8; Chaikoff, Reichert, Read and Mathes, 14), and after splanchnicotomy (Dresel and Omonsky, 15) and ergotamine (Miculicich, 16).

The question, in the present instance, is whether the effects of hypothalamic lesions as described here are manifested through the nervous system, are brought about through some influence upon the hypophysis, or both. The nervous factor cannot be ruled out in view of the results of experimental intervention in the more peripheral portions of the nervous system. How a derangement of hypophyseal function could be brought about is not clear. The present experiments advance no evidence that the posterior lobe, which is most intimately related to the hypothalamus, can be concerned. The lesions might influence the activities of the anterior lobe *a*, by injury to the site of action of certain of its hormones; *b*, by direct depressing influence upon the gland. The fact that some hypothalamic lesions appear to depress the diabetogenic activity of an anterior lobe suspension seems to lend support to the first of these propositions, and it will be recalled that according to Lucke (17) the site of action of this principle is within the central nervous system. However, the work of Houssay and Biasotti (18) with toads has led them to deny that anterior lobe hormones act by way of the hypothalamus, although they found that lesions in this region prevented or ameliorated pancreatic diabetes in this form. They ascribe the latter result to the loss of some direct nervous influence upon the anterior hypophysis. How hypothalamic lesions could cause such a loss is not clear, for the nervous connections between hypothalamus and anterior lobe, if any, are not yet understood. In this connection it should be mentioned that Davis, Cleveland and Ingram (19) report amelioration of pancreatic diabetes after hypothalamic lesions in the cat.

A completely satisfactory explanation of our results is not at present

forthcoming. Perhaps a more definite identification of the hypothalamic structures concerned will eventually aid in clearing up these matters. The chief comment on our lesions is that in order to influence carbohydrate metabolism they must be bilateral and situated either close to the walls of the ventricle in the region of the filiform nucleus or in the perifornical and subfornical regions of the anterior hypothalamic zone. The frequency with which atrophy or destruction of the filiform nuclei are associated with altered carbohydrate metabolism is striking, but here again exceptions may occur; for instance, cat 1, in which these cell groups were destroyed, responded in a normal manner to the hyperglycemic activity of anterior lobe suspension. The matter of more exact localization of the areas which appear to be concerned in some way with the regulation of carbohydrate metabolism must be said to await further study.

SUMMARY

1. Nine cats with bilateral lesions in the anterior, suprachiasmatic portion of the hypothalamus showed increased sensitivity to insulin as compared with normal controls. In these animals there were varying degrees of injury or atrophy of the filiform nuclei. Five other cats with lesions in the hypothalamus which did not affect this region so extensively showed normal responses to insulin. Three hypophysectomized cats also showed abnormal sensitivity to insulin.

2. Five cats with severe damage to the hypothalamus in the suprachiasmatic region showed a diminished response of the blood sugar to epinephrine.

3. Four out of 5 cats with lesions in the suprachiasmatic region failed to show normal responses to the hyperglycemic effect of a suspension of anterior hypophysis which was effective in normal cats. Three hypophysectomized cats showed retarded responses to the action of this preparation, but the results are not directly comparable since the dosage of anterior lobe was much less than in the 5 cats mentioned above.

4. The liver glycogen, determined just before the animals were killed, was normal in all the cats except one without a hypophysis, which had been under starvation conditions for several days.

5. Whether the disturbance in carbohydrate metabolism is due to a direct effect of the nervous lesions upon visceral structures or to an effect upon the hypophysis has not been determined.

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THE INTRINSIC REGULATION OF THE CIRCULATION IN THE PARIETAL CORTEX OF THE CAT

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The circulation in the surface (pial) blood-vessels of the parietal region of the cat has been extensively studied by Forbes and his co-workers (1928 et seq.) and an intrinsic regulation by nervous and chemical agents has been proved to exist. The physiological significance of such observations remains somewhat uncertain because the vessels in question are of relatively large size and are distinctly outside the brain; the vessels in intimate relation with brain cells may or may not respond like those on the surface. The thermoelectric method of Gibbs (1933) affords a means for studying the circulation within the substance of the brain. We have already (1934) used it in slightly modified form to study the circulation in the medulla and hypothalamus of the cat. This report deals with a series of experiments in which it was used in the parietal cortex.

The method and procedures were the same as those used in our preceding studies, the only essential difference being that the instrument was inserted into the cortex under the most prominent part of the parietal eminence. Cats narcotized with pentobarbital or chloralose and immobilized by curara were used exclusively. For nerve stimulation faradic currents were used. Control observations indicated that the results were due to changes in blood-flow and not to changes in body temperature or in local heat production, for when there was no heat gradient in the thermocouple no galvanometric deflections occurred upon carotid occlusion, adrenalin injection, CO_2 insufflation, or sympathetic nerve stimulation.

RESULTS. 1. *Vasomotor nerves.* The cervical sympathetic nerves were stimulated repeatedly in 22 valid experiments, the vagodepressors in 10, and the carotid sinuses in 6. The results of vagodepressor and carotid sinus stimulation were entirely negative: indicated blood-flow passively followed changes in blood-pressure, and never showed any signs of a direct vasodilator influence of these nerves upon the parietal circulation. Cervical sympathetic stimulation, however, caused a definite (more than 5 mm.) fall in indicated blood-flow without fall in blood-pressure in 20 of the 22 experiments, and the two negative results were obtained in animals in

which nerve stimulation was not attempted until after a long series of chemical influences had been tested. It is probable that these animals would also have shown positive vasomotor responses if the test had been made earlier in the experiment.

The results of cervical sympathetic stimulation in the 20 positive experiments are shown in figure 1. Each line represents a different experiment; the period of stimulation is shown by the unbroken line, the period of recovery by the broken continuation. The illustrated responses were chosen from a large number of observations because only these had the following characteristics which we regard as essential:

The thermocouple was imbedded in the parietal cortex for at least 1 mm., usually 2 or 3. Indicated blood-flow was steady during a control period of at least one minute. Blood-pressure either rose or showed no change during the periods of observation shown in the figure. Effective stimulation of the nerve was proved by the occurrence of retraction of the nictitating membrane or mydriasis or both. Pulmonary ventilation and rectal temperature remained unchanged during the periods of observation. Under such circumstances no similar changes in indicated blood-flow were ever observed to occur spontaneously.

The vasoconstriction produced in the parietal cortex by stimulation of the cervical sympathetic had the same characteristics that were observed in our experiments on the hypothalamus (Schmidt, 1934). There was a latent period, ranging from 5 seconds to a minute, averaging about 20 seconds, during which indicated blood-flow either rose or showed no change; this latent period was absent in only one of the 20 animals. The vasoconstrictor effect developed gradually during the stimulation, almost never reaching its maximum within one minute. After the stimulation the vasoconstriction usually continued to progress further for another minute or more. Recovery was entirely lacking in 14 of the 20 (70 per cent), partial, including any tendency in that direction, in 5, and complete in only one. The same characteristic latent period, slow progression, and tendency to persistence of the constrictor effect were found in the experiments on the hypothalamus. There were, however, the following points of difference:

First, the parietal response was more uniform. As already mentioned, there was definite parietal vasoconstriction on sympathetic stimulation in 20 out of 22 valid experiments, and the two negative results may well have been due entirely to loss of reactivity in the course of a long experiment dealing with chemical influences. In the hypothalamic experiments 10 out of 39 (26 per cent) showed no vasoconstriction on sympathetic stimulation.

Second, the threshold of the parietal vessels to cervical sympathetic stimulation was lower than was the case in the hypothalamus. In the latter

strong currents (secondary horizontal, 12 cm. or less from primary) were required, but in the parietal experiments much weaker currents were effective. Thus, in many instances there were quite marked changes in parietal blood-flow when the secondary coil was tilted to an angle of 60 degrees at 12 cm. from the primary, and in practically every case there were maximal effects with the secondary tilted at 30 degrees at 12 cm. distance. The weaker of these currents barely sufficed to cause mydriasis in some cases, although retraction of the nictitating membrane occurred. The threshold of the vessels of the tongue or temporal muscle was only a little lower than this (Schmidt, 1934).

Third, the vasoconstrictor fibers of one cervical sympathetic were more bilateral in their distribution in the parietal region than in the hypothalamus. In 7 valid experiments in which both nerves could be stimulated without attendant artefacts (fall in blood-pressure, etc.) there was parietal vasoconstriction on stimulating the contralateral nerve in every case.

Fourth, the cervical sympathetics evidently carry tonic constrictor impulses to the parietal region, for on firm ligation or section of the nerves indicated blood-flow in the parietal region rose definitely and consistently. The effect was of the same order as that produced by insufflation of 5 per cent CO_2, which means that it was relatively slight, but its consistent occurrence is noteworthy. In the hypothalamic experiments no corresponding effect was ever observed.

Fifth, in 4 of the parietal experiments weak stimulation of the sympathetic elicited a response that was mainly or purely vasodilator in nature. Stronger stimulation however elicited characteristic constrictor responses. We attribute the dilator responses to constriction of extracranial vessels by a current too weak to elicit distinct constriction in the parietal vessels; the threshold of the latter would therefore be somewhere between the two currents used in these instances (secondary at 12 cm. tilted at 60 and 30 degrees respectively).

All the foregoing points of comparison between the hypothalamic and parietal circulations suggest strongly that the vasoconstrictor innervation is more direct, more abundant, and more effective in the parietal region. The features which led us to suggest (Schmidt, 1934) that a humoral mechanism may be involved in the hypothalamic response are likewise present in the parietal response, namely, slow onset, slow development, and very slow recovery.

We have also attempted to demonstrate a direct and potent connection of the parietal vessels with the vasomotor center, as in our earlier experiments on the hypothalamus, by stimulating the center chemically (by asphyxia or strong CO_2) and electrically (through wires introduced into the medulla and the roof of the mouth or the cervical cord). The results were again entirely negative: the chemical stimulants caused pure increases

in parietal blood-flow, and the electrical stimulations had no effect unless blood-pressure was altered, in which case the blood-flow curve followed the change faithfully and apparently quite passively.

Stimulation of the vagodepressor and carotid sinus nerves never elicited anything but passive effects in the parietal region. This was equally true in the hypothalamus. We have not yet succeeded in demonstrating

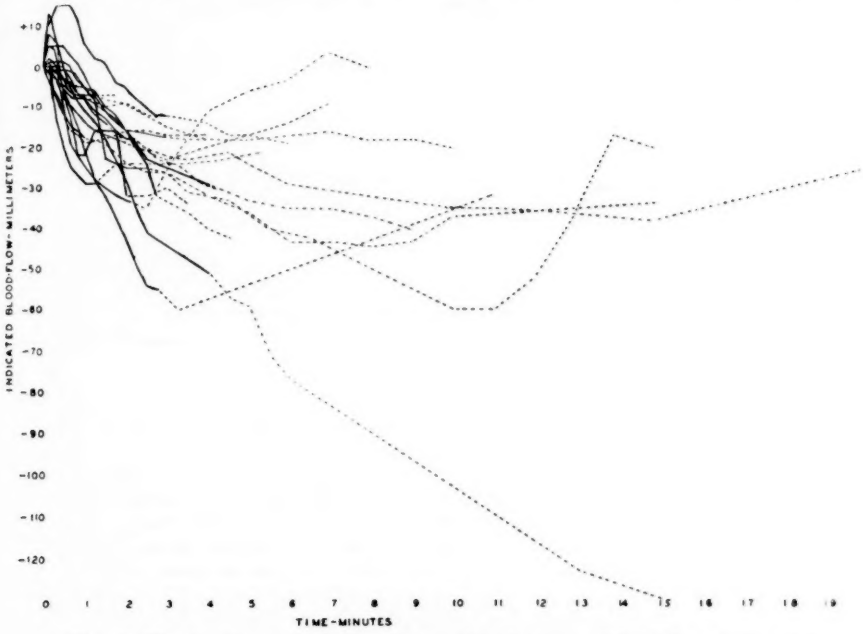

Fig. 1. Effects of cervical sympathetic stimulation on blood-flow in parietal cortex.

From 20 experiments on cats under pentobarbital or chloralose, curara, and artificial respiration. Instrument imbedded in parietal cortex in each case. Period of stimulation is shown as the unbroken line, period of recovery by the broken continuation; each line represents a different experiment. Strength of stimulation usually 12 cm.—30 degrees.

vasomotor influences, constrictor or dilator, exerted by these nerves upon any part of the cerebral circulation.

The response of surface (pial) vessels. Up to this point we have been dealing exclusively with experiments at the end of which the thermocouple was found to have been definitely imbedded in the substance of the brain for a distance of at least one millimeter. In a number of additional experiments the thermocouple either by accident or design came to rest upon the

surface of the brain without penetrating it. In these cases the result of sympathetic stimulation was almost invariably of the rapid extracranial type instead of the delayed, slowly developing vasoconstriction that was always encountered when the recording instrument was surrounded by brain tissue. Figure 2 shows the result of cervical sympathetic stimulation in 9 experiments in which the thermocouple had perforated the dura

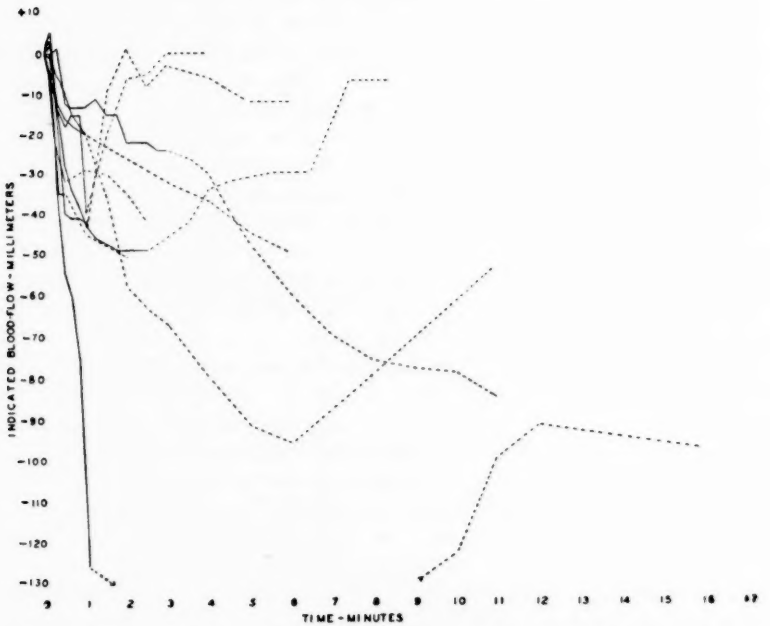

Fig. 2. Effects of cervical sympathetic stimulation on blood-flow in surface (pial) vessels of parietal cortex.

From 9 experiments on cats prepared as usual (fig. 1) except that thermocouple was in contact with surface of parietal lobe without penetrating it. Duration of stimulation 1 minute in all but two; in one it was 3 minutes, in the other 30 seconds; this period is shown as an unbroken line, the recovery period as the broken continuation. Strength of stimulation 12 cm.—30 degrees in all cases. Each line represents a different experiment.

and established contact with the surface of the parietal lobe without penetrating it. The scale and mode of portrayal are the same as in figure 1, as are also the criteria for selection (stable control period, absence of fall in blood-pressure, constancy of alveolar ventilation and rectal temperature, positive ocular effects). Each line represents a different experiment.

The differences resulting from the superficial position of the instrument

were as follows: First, the onset of the vasoconstrictor response was much more rapid, with only one exception, in which there was a latent period of 20 seconds, or about the average for the responses of the parietal cortex (fig. 1); there was no latent period whatever in 3 animals, and in the remaining 5 it was less than 10 seconds.

Second, the effect progressed more rapidly, again with the same single exception, and the lines of figure 2 therefore are more nearly vertical than those of figure 1.

Third, there was more tendency to recovery, which was complete in 3

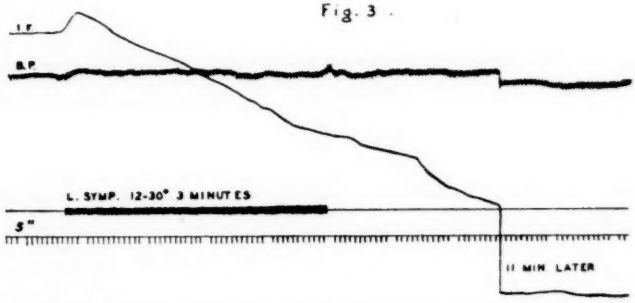

Fig. 3. Effect of cervical sympathetic stimulation on blood-flow in parietal cortex. Cat—pentobarbital, curara, artificial respiration. Instrument in left parietal cortex—penetration about 2 mm. Both sympathetics tied firmly but not cut; left stimulated for 3 minutes with secondary 12 cm. from primary and tilted at 30 degrees. Latent period 10 seconds, then rise of 9 mm. in indicated flow during next 10 seconds; blood pressure first rose from 148 to 152, then came down to 150, where it remained during stimulation and for 3 minutes thereafter, at which time indicated flow was 81 mm. below its control level. During pause of 11 minutes indicated flow fell further to 122 mm. below its control level, with blood pressure 140 mm.

In this and subsequent records the time tracing indicates 5 second and 1 minute intervals and is the zero level for the blood-pressure record. All records to be read from left to right. The original tracings have been copied over a transparent plate.

and partial in 2 others, so that some recovery took place in 5 of the 9 experiments, compared with 6 of the 20 shown in figure 1.

Fourth, the vasoconstriction seemed to be more intense, for the galvanometer showed greater deflections in these experiments.

These differences are well shown by a comparison of the greatest effects produced by cervical sympathetic stimulation with the thermocouple in the substance (fig. 3) and on the surface (fig. 4) of the parietal lobe. The behavior of the surface vessels is quite like that of extracranial vascular areas (Schmidt, 1934); although latent periods were more frequent, the indicated vasoconstriction was usually less intense, and in one case there was a slow intracranial type of response. It seems fair to summarize by

saying that the response of the surface vessels is roughly intermediate between that of the cortical circulation on the one hand, and that of extracranial vascular areas such as the temporal muscle and tongue on the other.

In this interpretation one obvious basis for deception is the fact that in the surface position there was no injury to the vascular system under investigation, whereas in all our other experiments trauma from insertion of the instrument was unavoidable. That the insertion of our thermo-

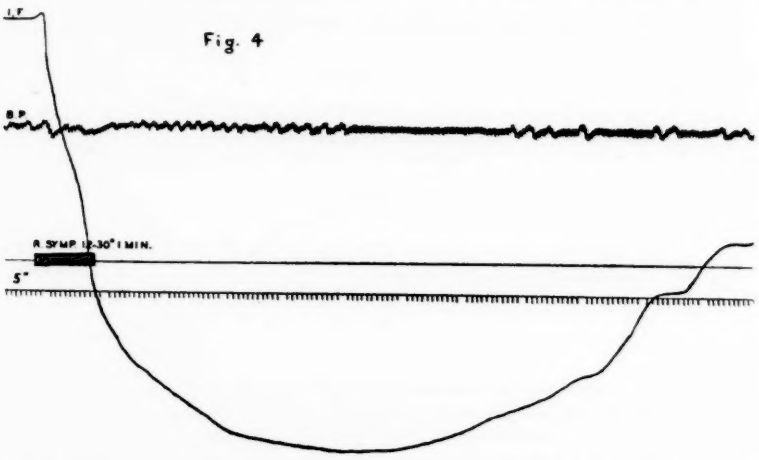

Fig. 4. Effect of cervical sympathetic stimulation on blood-flow in surface (pial) vessels of parietal region.

Cat—pentobarbital, curara, artificial respiration. Instrument in right parietal region; hard-rubber guide 2 mm. longer than usual, so that tip of thermocouple was in contact with the surface of the parietal lobe without penetrating it (position confirmed at end of experiment). Right sympathetic tied firmly but not cut; stimulation with secondary at 12 cm. from primary and tilted to 30 degrees, for one minute. Indicated flow rose 3 mm. during the first 5 seconds, then fell sharply to reach a minimum (total fall 217 mm.) at 4 minutes after the end of the stimulation; it recovered to a point 92 mm. below the control level by the end of the next 8 minutes, but rose no further during the succeeding 6 minutes.

couple into a vascular area does not in itself produce the conditions responsible for the slow onset, progression, and recovery that we have regarded as characteristic of intracranial vasoconstriction of sympathetic origin, is shown by the results obtained in extracranial tissues (Schmidt, 1934). There was nothing slow or sluggish about those responses although the trauma inflicted upon the tissue was at least equal to that to which the brain was subjected. To prove that cerebral vasoconstriction of sympathetic origin is slow in the absence of trauma, we inserted the thermocouple

into the sagittal sinus, and in 4 experiments succeeded in getting the tip of the instrument either directly on its outer wall or inside its lumen, without hemorrhage, thrombosis, or contact with the brain itself. The preparation was otherwise the same as in the other experiments (pentobarbital, curara, etc.).

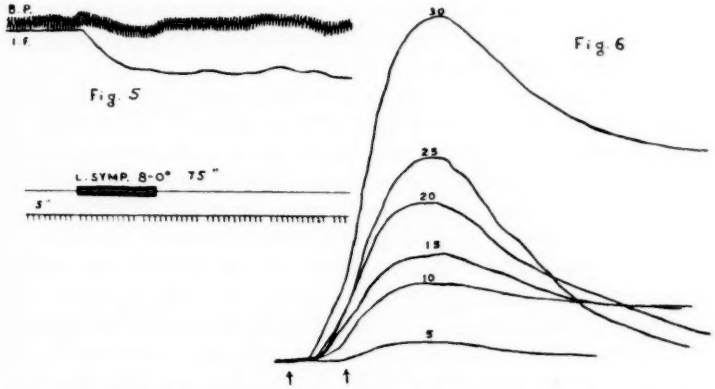

Fig. 5. Effect of cervical sympathetic stimulation on blood-flow in sagittal sinus.

Cat—pentobarbital, curara, artificial respiration. Tip of instrument in lumen of sagittal sinus—position confirmed after experiment, when free bleeding occurred from hole left by withdrawal of instrument. Left cervical sympathetic ligated firmly but not cut; stimulation with secondary horizontal, 8 cm. from primary, for 75 seconds.

Fig. 6. Effects of insufflation of ascending concentrations of CO_2.

Cat—pentobarbital, curara, artificial respiration. Instrument in left parietal cortex. CO_2 in air applied to pump intake in concentrations of 5, 10, 15, 20, 25, and 30 per cent, each for one minute (between the arrows); the original records are superimposed.

Effects on blood-pressure:

5 per cent CO_2—control level 152 mm., maximum 162 mm., recovery to 144 mm.
10 per cent CO_2—control level 134 mm., maximum 152 mm., recovery to 152 mm.
15 per cent CO_2—control level 152 mm., maximum 172 mm., recovery to 160 mm.
20 per cent CO_2—control level 154 mm., maximum 178 mm., recovery to 160 mm.
25 per cent CO_2—control level 160 mm., maximum 190 mm., recovery to 170 mm.
30 per cent CO_2—control level 170 mm., maximum 192 mm., recovery to 170 mm.

The results obtained in 8 sympathetic stimulations in these 4 animals were not striking but they were consistent, and they were like those obtained when the instrument was imbedded in brain substance, not like those recorded when it was on the surface. The latent period, always present, ranged from 10 to 40 seconds; the effect progressed slowly to reach a maximum by the end of the stimulation (75 and 60 seconds) in two cases, while in all the others it continued to progress for 30 seconds or more after

the stimulation. The most striking of the responses is shown in figure 5. It looks exactly like one of the less marked effects recorded with the thermocouple imbedded in the parietal cortex (fig. 1) and not at all like those observed when the instrument was touching the surface of the brain (fig. 2).

As far as we have been able to determine, therefore, the differences between figures 1 and 2 are due to inherent differences in the responses of cortical and superficial (pial) vessels to sympathetic nerve stimulation, and the former evidently gives the more accurate picture of the behavior of the circulation within the brain.

2. *Chemical influences.* These experiments followed the same general plan as those on the medulla and hypothalamus. The animals were narcotized with pentobarbital and immobilized with curara, additional doses of which were given whenever signs of respiratory activity appeared. Attention was concentrated upon the effects of increase and decrease in CO_2 and oxygen content of the blood; in a few cases fixed acid and alkali were injected. Adrenalin, pituitrin, histamine, acetyl choline, and nitroglycerine were tested both by intravenous and intra-arterial injection.

The results of changing the CO_2 and oxygen content of the blood were very similar to those obtained in the hypothalamus. Increases in blood CO_2 caused step-like increases in parietal blood-flow, and decrease in blood CO_2 (by increased ventilation) caused decrease in the blood-flow in the face of a rise in blood-pressure. Increase in blood oxygen caused vasoconstriction and anoxemia (nitrogen insufflation) caused vasodilatation.

Examples of these effects are shown in figures 6, 7, and 8. Since they are so similar to those previously reported in the medulla and hypothalamus additional details seem unnecessary. Compared with the hypothalamus the parietal region responded about the same to the dilator effect of increased CO_2 and somewhat more intensely to the constrictor effect of decreased CO_2; there was no sign of an upper limit here, as there was in the hypothalamic experiments. Increased oxygen had much more constant and marked effects in the parietal region than in the other areas. The dilator effect of maximum anoxemia was always inferior to that of insufflation of strong CO_2, but it was greater than in either of the other areas. Fixed acid (HCl, ammonium chloride) had no consistent or striking effects and alkali (sodium bicarbonate) only increased parietal blood-flow.

Because the cervical sympathetic nerves seemed to have a more direct and marked influence on the parietal circulation than on that of the other investigated areas of the brain, we tested the influence of severing the nerves upon the responses to chemical agents. The results were entirely negative: the effects of changes in blood CC_2 and oxygen were practically identical before and after the nerves were cut. Since this was the case in 4 satisfactory experiments the subject was not pursued further.

With the other agents the results were in some respects quite unlike those

obtained in the hypothalamus. Adrenalin never caused any vasoconstriction in the parietal region, even when it was injected directly into the carotid artery on the side on which the instrument was inserted; in the hypothalamic experiments there was frequently vasoconstriction before

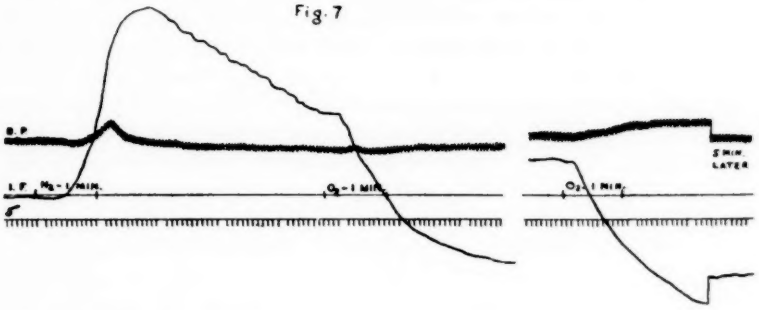

Fig. 7. Effects of anoxemia and of oxygen insufflation.

Cat—chloralosane, curara, artificial respiration. Instrument in right parietal cortex. Nitrogen (100 per cent), followed by oxygen, by way of intake of air pump, each for one minute. Second record—later in same experiment; oxygen for one minute during adequate ventilation with air.

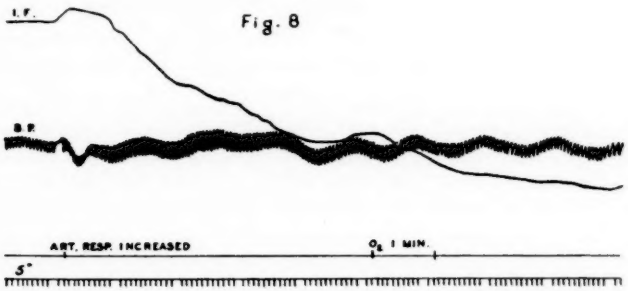

Fig. 8. Effects of increased ventilation and of oxygen insufflation.

Cat—pentobarbital, curara, artificial respiration. Instrument in left parietal cortex. Artificial respiration increased by increasing thrust of air pump, rate remaining unchanged except for brief pause during the readjustment. Oxygen applied through intake of air pump for one minute after maximal decrease in flow had been obtained from the increased ventilation.

and after the rise in blood-pressure produced by the drug. Pituitrin, injected into the carotid in 0.1 to 0.2 cc. dosage, had the surprising effect of producing quite marked parietal vasodilatation in each of two animals in which it was tested; there was only an insignificant rise in blood-pressure, and the dilator effect was much greater than that of adrenalin, histamine,

or any other agent tested excepting strong (30 per cent) CO_2 or pure nitrogen. Histamine produced much less vasodilatation in the parietal region than in the hypothalamus, but there was a brief dilator response as blood-pressure was recovering from the depressor effect of the injection. The same was true of acetyl choline, which was decidedly more effective than histamine in this respect. Nitroglycerine was still more effective, the dilator response being more marked to this drug than to any of the others excepting pituitrin.

Examples of these responses are shown in figure 9. In addition ether insufflation was tried in 3 experiments and morphine injection (intramuscularly) in 4 others. Ether caused a definite though relatively slight

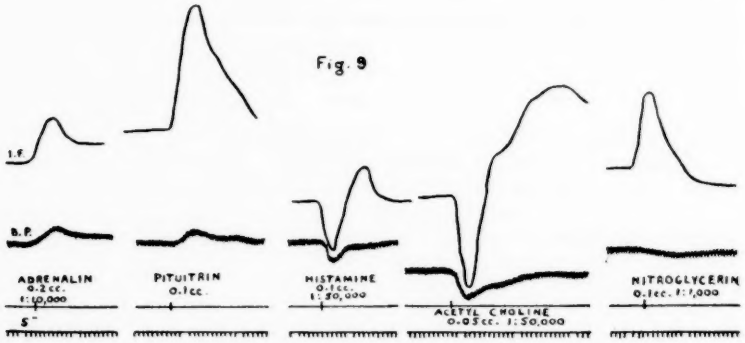

Fig. 9. Effects of intracarotid injections of adrenalin, pituitrin, histamine, acetyl choline, and nitroglycerine.

Cat—pentobarbital, curara, artificial respiration. Thermocouple in left parietal cortex. Injections made through a needle tied into the thyroid branch of the left common carotid. All results from the same experiment.

increase in parietal blood-flow without rise in blood-pressure, but morphine had no definite effect.

Discussion. These experiments have further strenghthened the conviction derived from previous work (1934) that generalizations concerning the cerebral circulation as a whole cannot properly be drawn from observations made upon any one part of it. Since the cervical sympathetic innervation appears to be more intimately concerned with the physiological regulation of the circulation in the parietal region than in the hypothalamus, the question of the physiological significance of the vasoconstrictor innervation of cerebral vessels becomes much more complicated. The same is true of the chemical regulation. We are still able to say that the vasodilator effect of CO_2 is the most potent single influence upon all parts of the cerebral circulation and that an intrinsic regulation through this agency

is probably the chief factor in the normal regulation of the cerebral circulation as a whole. But oxygen insufflation produced vasoconstriction in the parietal region more consistently and more intensely than in the medulla or hypothalamus. Furthermore, the effects of adrenalin, pituitrin, histamine, and choline derivatives show evidences of even greater complexity. Adrenalin, weakly vasoconstrictor in the hypothalamus, had no detectable direct influence in the parietal region, so that the humoral element in the transmission of sympathetic impulses to cerebral vessels, if it exists, cannot be identical with adrenalin, and sympathetic vasoconstrictor control does not run parallel to the response to adrenalin.

Our observations on the circulation in the parietal cortex confirm, in all essential respects but one, the results of direct microscopic observation of the pial circulation in this region of the cat's brain, as practised by Forbes (1928) and his collaborators. This includes vasoconstriction on stimulation of the cervical sympathetic (Forbes and Wolff, 1928; Cobb, 1929; Pool, Forbes, and Nason, 1934); vasodilatation from increased CO_2 or decreased oxygen in the blood, vasoconstriction with decreased CO_2 or increased oxygen, with the CO_2 influence far the more powerful (Wolff and Lennox, 1930); vasodilatation with histamine (Forbes, Wolff, and Cobb, 1929), acetyl choline (Wolff, 1929), pituitrin and adrenalin (Forbes, Finley, and Nason, 1933). The one major discrepancy is our failure to demonstrate any vasodilator innervation *via* the vagodepressor, as reported for the pial circulation by Forbes and Wolff (1928) and Cobb and Finesinger (1932), or *via* the carotid sinus mechanism, as reported by Ask-Upmark (1934). We are inclined to ascribe the difference to our routine use of curarized animals, thus obviating the changes in pulmonary ventilation and intrathoracic venous pressure that are likely to result from stimulation of these nerves—changes which, in our experience, are not prevented by artificial respiration alone, and which might simulate a vasodilator innervation that is actually not present. Finesinger and Putnam (1933), in experiments in which the brains of monkeys and cats were perfused with their own blood by way of one carotid, found evidence of cerebral vasodilatation on stimulating the central ends of the vagodepressor nerves; the vasodilatation was manifested in an increased perfusion flow without change in perfusion pressure. As far as the monkey is concerned, we have no corresponding observations. In the cat, however, anastomoses between the external and internal carotid systems are so abundant that an indicated change in the perfused carotid circulation cannot be interpreted as evidence that the change was intracranial—a fact of which Finesinger and Putnam were well aware, and which they circumvented by direct observation of pial vessels in 6 animals; of these only one showed cerebral vasodilatation on stimulation of the vagodepressor nerves.

While it is true that we have succeeded in confirming, in nearly all

respects, the results of observations made on the pial circulation, we have also been able to show that the behavior of the pial vessels is not entirely indicative of the behavior of vessels in the subjacent cortex. The pial response to sympathetic stimulation is more rapid, more reversible, and probably more vigorous than that of the cortical vessels; it is more like that of extracranial than intracranial vessels; it does not reveal the latent period, slow progression, and persistence that appear to be characteristic of the vasoconstrictor response of the cerebral circulation to sympathetic nerve stimulation.

SUMMARY AND CONCLUSIONS

The circulation in the parietal cortex of anesthetized, curarized cats was studied by a thermoelectric method used in previous similar studies in the medulla and hypothalamus.

Vasoconstrictor innervation *via* the cervical sympathetic was constantly demonstrable; the response to stimulation of this nerve was slow in onset, development, and recovery. Section of the nerves regularly caused vasodilatation in the parietal region. No vasodilator innervation could be detected by stimulation of the vagodepressor and carotid sinus nerves.

Changes in CO_2 and oxygen content of the blood affected parietal blood-flow in the same way as that of the other cerebral areas already investigated: increased CO_2 and decreased oxygen caused vasodilatation, decreased CO_2 and increased oxygen caused vasoconstriction. The effects of oxygen were more intense in the parietal region than in the other areas. Fixed acid and alkali had no detectable effects.

Adrenalin had no vasoconstrictor effect in the parietal region; pituitrin, histamine, acetyl choline, and nitroglycerine were vasodilator. In the hypothalamus adrenalin was weakly vasoconstrictor and pituitrin had no apparent effect.

The vasoconstrictor innervation of the parietal circulation differs from that of the hypothalamus in lower threshold to faradic stimulation, maintenance of a tonic control, and greater consistency in its effectiveness. Presumably the physiological significance of the innervation is different in the two regions. This, together with the greater reactivity of the parietal vessels to changes in blood oxygen and the differences in the effects of adrenalin and pituitrin in the two regions implies that the intrinsic regulation of the cerebral circulation is more complex than has been supposed hitherto.

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THE TIME-INTENSITY CURVE AND LATENT ADDITION IN THE MECHANICAL STIMULATION OF NERVE

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The almost exclusive use of electrical stimulation as a tool in the attempt to elucidate the nature of the excitatory processes in tissue is attended with the danger that it engenders a point of view which may be too electrical, so that there are brought forth explanations which make great use of condenser, and other similar electrical analogies, which perhaps on the whole have not much probable validity. For this reason it is desirable to test other types of stimuli which will yield data of a similar nature, i.e., giving time-intensity relations, the analysis of which will permit and suggest other quite different explanations of excitation phenomena.

Stimuli other than electrical do not, on the whole, on account of their nature, lend themselves well to the obtaining of time-intensity data. Chemical stimuli, for example, entail a process of diffusion in reaching their place of action and they must be dissipated again before the tissue returns to normal. Mechanical stimuli appear in fact to be the only ones other than electrical which promise to give results of any value for the purpose.

It was shown long ago by Tigerstedt (1882) that mechanical stimuli to frog's nerve are relatively uninjurious. He used a dropping weight method (1881) by means of which he was able to stimulate the same nerve trunk several hundred times. The difficulty of measuring the time of application of any solid stimulators seems to preclude their use. Jets of air do not, however, suffer this disadvantage to the same extent. The introduction of air jets as stimuli appears to be due to Allen and Hollenberg (1924) who used them to investigate the touch and pressure organs in the skin. Their data were concerned with the fusion frequency of intermittent stimuli rather than with time-intensity relations. Their work suggested, however, a trial of air jets for the mechanical stimulation of nerve trunks. The present work deals with the results of this procedure.

APPARATUS AND METHOD. The first apparatus used was an analogue

of the electrical condenser stimulator. It consisted of a glass cylinder measuring 2.5 inches in diameter and 15 inches in length which was connected by tubing to a water reservoir. Water could be introduced into or removed from the glass cylinder so that its capacity could be altered at will. At the top of the cylinder were three outlets leading by rubber tubes, one to the tissue, one to a mercury manometer, and one to the compressed air line. When the tube leading to the tissue was closed the cylinder having any required capacity could then be blown up to any desired pressure. A quick release mechanism on the tube leading to the tissue could then be activated, allowing the air in the cylinder to escape and in doing so to impinge upon the nerve trunk which was held in one of a number of arrangements which will be described later. The stream of air emerging from the cylinder was of long duration when the capacity was large and of short duration when the capacity was small. The initial intensity of the stream depended upon the initial pressure. The intensity died away in a manner roughly analogous to exponential decay, as was ascertained by photographing the movement of a membrane manometer communicating with the cylinder.

This device, while simple and easily constructed, did not give data easily analyzable on account of the difficulty of expressing the forms of the stimuli as equations. It was considered more practical, therefore, to construct an arrangement for delivering air pulses of constant intensities and known durations suitable for obtaining the analogue of the direct current strength-duration curve.

This apparatus consisted of a brass slot 6 by 1 by $\frac{1}{16}$ inches, into which was fitted each of a set of brass slides 12 inches long. The sides of the slot were held in place by springs so that they fitted closely to the slides. On opposite sides of the slot, 2 inches from one end, were tranverse rectangular apertures $\frac{1}{16}$ by $\frac{5}{16}$ inch. One of these led into a tube connected to a supply of compressed air; the other led to a tube at the end of which the tissue was placed. In each slide was a rectangular aperture also $\frac{5}{16}$ of an inch wide. In the set of slides the lengths of these apertures ranged from $\frac{1}{8}$ inch to 1 inch. By means of a heavy weight and pulley arrangement any one of the slides could be pulled rapidly through the slot. Thus, for a given duration depending, apart from the speed of the falling weight, only upon its length, the aperture in the particular slot used permitted air to pass from the source of supply to the tissue.

The air supply was obtained by connecting the house system to a 20 liter bottle, thence to the slot arrangement. A mercury manometer and a T-tube were connected to the latter circuit. By holding the finger or a suitable valve on the open end of the T-tube the pressure could be built up to any desired level and maintained during an excursion of the slide. The pressure was then released for the return of the slide.

The durations of the pulses were measured by photographing the rotation of the pulley while the weight fell. The resulting times, on account of the friction in the arrangement, were somewhat longer than those calculated, considering the weight to be falling freely.

Various arrangements for holding the preparation were used. The one in which the nerve trunk was most easily stimulated consisted of a glass T-tube, the horizontal part of which was 2 cm. in length and 1.5 mm. in internal diameter. The vertical part, of larger tubing, was connected to the stimulator. The nerve trunk was drawn through the horizontal tube preparatory to stimulation. During stimulation, the air stream divided so that it left both ends of the tube and in doing so it probably exerted a stretching action on the preparation. This particular arrangement was not very satisfactory as the nerve trunk was frequently displaced or caused to flutter by the air stream.

A satisfactory arrangement consisted in allowing the air to emerge from a brass tube which had been flattened at the end to a slit about 0.3 mm. in width and 1 cm. in length. The nerve trunk, which was set up in a groove in an elliptical bar of hard rubber, was placed at right angles to the length of the jet and separated from it by about 2 mm. The stimuli in this case did not displace the preparation and the data obtained were, in consequence, more consistent. Pressures several times greater were needed, however, for stimulation.

With either of these arrangements the injury to the tissue seems to be quite small. This has been shown by using the same preparation on three different occasions during a week, several dozens of stimuli being applied each time to the same stretch of the nerve. The air, of course, has a drying action so that Ringer's solution must be applied after each stimulus. This drying may be more detrimental to the tissue than the stimuli themselves.

EXPERIMENTAL RESULTS. *Condenser analogue.* In figure 1 is given a typical pressure capacity curve for the sciatic nerve of *Rana pipiens* using the device analogous to the condenser. The abscissae, the capacities, are arbitrary, but the ordinates, the pressures, are in centimeters of mercury. It will be observed that the curve is similar in form to the voltage-capacity curve using electrical stimuli. The minimal pressure which is approached asymtotically is in this case about 5 cm. of mercury and such a value is quite common with the T-tube arrangement. Pressures much lower than this are seldom effective so that the pressure which is analogous to the rheobase for electrical stimulation is usually 5 cm. or more of mercury. It will be convenient to call this quantity the pressure rheobase. The curve of figure 1 attains about three of these rheobases with the lowest capacity. As was remarked above, the timing of these stimuli is difficult so this aspect will be considered in connection with the rectangular pulses.

Rectangular pulses. In figure 2 is given a pressure-time curve of the sciatic nerve of the frog for the rectangular pulses, the pressures being in centimeters of mercury and the times in milliseconds. In this case the wide jet was used. It will be observed that this also has a form similar to that of the electrical strength-duration curve. The shortest duration obtainable was 2 milliseconds which requires only about two rheobases, so the curve is short. Pairs of readings, not consecutive, are given for the shortest and longest durations. These indicate the variations of the readings, which are frequently between 5 and 10 per cent, so that the accuracy is considerably less than with electrical stimuli. It will be observed that the analogue of chronaxie in this case is between 1 and 2 milliseconds. This is a typical value which is considerably greater than those obtained

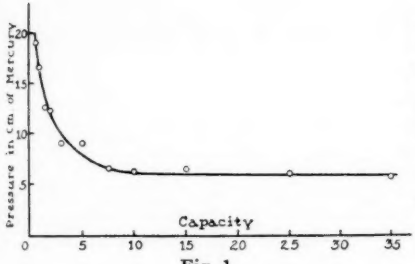

Fig. 1

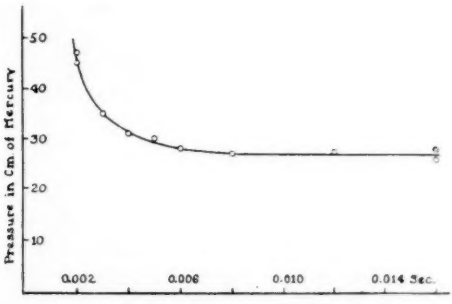

Fig. 2

Fig. 1. The pressure-capacity curve for the sciatic nerve of the frog at 23°C. using the arrangement analogous to an electrical condenser stimulator.

Fig. 2. The strength-duration curve for the sciatic nerve of the frog at 23°C. using rectangular air pulses.

with electrical stimuli. The possible significance of this difference will be discussed later.

Latent addition. In table 1 is given a typical set of data on latent addition. These data were obtained with the same apparatus except that a slide with two apertures instead of one was used. The first row gives the pressures required for stimuli of 0.002 second duration; the second row gives the pressures required for two successive stimuli of 0.002 second duration with an interval of 0.002 second between them. No other procedure was used as the apparatus did not allow the use of shorter intervals. It will be observed that the pressures required with the two stimuli are less but not greatly less than with one. There is summation, therefore, of the inadequate stimuli but the excitatory state due to the first has become almost dissipated by the end of the 0.002-second interval. There was some variation with different preparations but the pairs of stimuli

ranged in value from about 85 to 95 per cent of the single stimulus, so it seems permissible to conclude that the subsidence of the inadequate excitatory state is almost complete after 0.002 second.

Discussion. One respect in which the reactions of tissue to different kinds of stimuli should be particularly informative is in the dissociating of those parts of the excitatory process which are purely physiological from those which are consequent to the stimulus directly. For, in electrical stimulation, it is well established that during the action of a stimulus there are at least two processes involved: a building up of the excitatory state, and a simultaneous spontaneous subsidence of the excitatory state which is also, of course, the determining factor in latent addition. This latter process, if it exists with other stimuli and is the same, may be considered purely physiological in that it constitutes the tendency of the tissue to recover its normal resting condition independently of what means were used to produce its state of excitation. In any physical theory of excitation the subsidence process must be introduced in a certain way. Where it is assumed, for example, that the process of excitation is due to

TABLE 1

Single	33	32	34	32	32	31	32
Double	29	28	28	28	28	28	27

The pressures in centimeters of mercury required for single stimuli of 0.002-second duration (upper row) and for double stimuli of the same durations separated by 0.002 second (lower row). The readings in each column were successive.

the accumulation of ions such as in Nernst's theory (1899) or Hill's (1910), the subsidence is the spontaneous redistribution of these ions; where it is assumed that the process of excitation is due to the charging of a leaky condenser (Lapicque, 1907) the subsidence is the spontaneous leaking of the condenser, and so on. These particular theories were, of course, suggested by the fact that electrical stimuli were used and they may not fit in well with any ideas which seem probable with regard to excitation by mechanical stimuli. It is possible, of course, that mechanical stimulation may produce an excitatory state of a different kind from that produced by electrical stimulation. This is not probable, however, and it will be of interest to see if the data will indicate the existence of a subsidence factor of the same kind as that given by electrical data and if it does, it will be strongly indicative that the same kind of a state of excitation is produced by both types of stimuli.

As regards the building of the the state of excitation it can scarcely be expected that two different kinds of stimuli will produce the same end result in the same way even though the same constituents of the tissue are involved in the process. It can scarcely be expected, for example,

that an electric field will operate upon the components of the excitable structure in quite the same way as does a mechanical stress even though both achieve the same final result. In the differential equation for the growth of the excitatory process it does not seem probable, therefore, that the pressure can be introduced in the same way as the potential.

In the electrical case it has been shown that the time-intensity data can be correlated adequately in terms of solutions of the differential equation (Blair, 1932; 1935)

$$\frac{dp}{dt} = KV - kp \tag{1}$$

where p is the excitatory process, V is the applied voltage or current and K and k are constants. The fact that these solutions apply equally well to several different forms of electrical stimuli indicates that the differential equation itself is valid in very close approximation at least. In line with the discussion above it is permissible perhaps in the mechanical case to write, therefore,

$$\frac{dp}{dt} = F(P) - kp \tag{2}$$

where $F(P)$ is some as yet unknown function of the pressure and possibly the time and the excitatory state as well and k is the same parameter in the subsidence process as is encountered with electrical stimuli. The main problem of mechanical stimulation from the phenomenological point of view is the determination of the nature of this $F(P)$ providing the assumptions above are valid; if not, that is, if the excitatory state in this case is totally different from that produced by electrical stimuli, the problem must be started at the beginning. In either case it will be better to defer attempts to solve this problem until data have been obtained which extend the time-pressure curve to at least several rheobases.

It will be convenient, meanwhile, if some deductions can be made from such factors as latent addition, chronaxie, and the rheobase. The data given here on latent addition are sufficient only to show the existence of the phenomenon and to enable an estimate of the time required for the subsidence of an almost adequate local excitatory state. The existence of the phenomenon permits only the conclusion that the inadequate states of excitation brought about by either mechanical or electrical stimuli persist for some time after the removal of the stimuli. The fact that the time required for these states to subside to a small fraction of their initial values is about the same for both stimuli suggests, however, that the states of excitation are the same.

In regard to the chronaxie it cannot be expected that the current and pressure chronaxies will be the same for the same tissue unless the equa-

tions of the pressure-time curve and the current-time curve are the same, which would result only if P and V had the same form in equations like (1) and (2) and the limits of integration were the same. As was remarked above this is not probable so that no particular significance can be given at present to the fact that the pressure chronaxie appears to be longer than the electrical.

With regard to the rheobase the simplest interpretation which can be given to the approach of a curve to such a lower limit is that the process giving rise to it is represented by an equation such as (2) which has reached the limit

$$\frac{dp}{dt} = O = F(P) - kp$$

where p is now the least adequate value for a response. The existence of a rheobase indicates therefore that a relation like equation (2) is correct, fulfilling the expectation that the subsidence factor evidenced by the latent addition data is also active during the application of the stimuli just as it is during electrical stimulation.

Another basis for the assumption that the same excitatory states are produced by electrical and mechanical stimuli is given by the results of Tigerstedt (1882) who, on investigating Pflüger's generalization on electrotonus, came to the conclusion that they were valid for mechanical stimulation also. That is, when tested by mechanical stimuli, the frog's sciatic nerve subjected to a constant current became more easily excited near the cathode and less near the anode and so on in a manner quite similar to that found on testing with electrical or chemical stimuli. That mechanical effects add algebraically to electrical effects to give the same results as adding electrical effects suggests strongly that they are the same things.

Taking together the findings of Tigerstedt, the form of the pressure-duration curve, and the existence of latent addition with mechanical stimuli, it appears probable that the states of excitation produced by mechanical and electrical stimuli are the same. If they are the same, it follows that their rates of subsidence will be the same. Therefore in any physical theory of excitation the subsidence factor must be given a meaning which can be consistent for both mechanical and electrical stimuli. It appears scarcely likely that existing hypotheses in which the redistribution of accumulated ions, or the loss of charge by a condenser are used for this purpose can be correct, but in view of the fact that the tissue is known to contain solutions with different concentrations inside and outside the cells it cannot be held that a transportation of fluid resulting in an accumulation of ions is impossible.

From the mechanical point of view alone it is more direct to suppose

that the state of excitation consists in the straining of a molecular arrangement, particularly so, since the T-tube apparatus, in which the nerve was probably stretched as well as compressed, required smaller pressures. This indicates that a longitudinal stress on the nerve fiber at a particular locus is adequate to bring about the excitatory state and if this process consists in the disarrangement of a molecular structure it is easy to imagine that a similar condition might be brought about by the action of an electric field.

The finding that mechanical stimuli cause little injury to nerve indicates that the receptor organs for these stimuli need not be highly differentiated. If they are like nerve it may be that they are stimulated easily on bending as this would usually stretch the cell at some point.

With regard to the possibility of nerves within the intact animal being stimulated by the blood pressure, it will be observed that the pressure rheobase with the one arrangement was as low as 5 cm. of mercury which is a possible value for the pulse pressure. Except in the case of the special pressure receptors, however, the apposition of arteries and nerves is probably such in general that much greater pressures would be required; and in any case greater mechanical stimuli may be required for these nerves.

SUMMARY

The sciatic nerve of *Rana pipiens* was excited by means of air jets using either rectangular pulses of given durations or pulses decaying approximately exponentially. Pressure-duration curves analogous to the electrical strength-duration curves were thus obtained. The existence of latent addition with mechanical stimulation was demonstrated by means of pairs of stimuli with a short interval between them. It is concluded that the results indicate that the states of excitation produced by both mechanical and electrical stimuli are the same and the consequences of such a view are discussed with respect to physical theories of excitation.

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THE EFFECT OF CARBON ARC RADIATION ON BLOOD PRESSURE AND CARDIAC OUTPUT[1]

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Considerable clinical and experimental evidence has accumulated to show that carbon arc radiation of sufficient intensity and duration lowers blood pressure (1). The changes in blood pressure, pulse rate and blood volume have been definitely established for the dog by Laurens and Mayerson (2), (3). Using the pulse rate-pulse pressure product as an indirect (and admittedly approximate) criterion, they calculated that there was a decrease of about 20 per cent in cardiac output following irradiation with a return to normal in about 24 hours. In the human, while blood pressure can be lowered quite readily in some hypertensive individuals, the depression is not as marked or is absent altogether in subjects with a normal pressure (1). Pulse rate changes are variable and probably due to heating. The only direct measurements of the influence of radiation on cardiac output are those of Lindhard (4) who found the minute volume output to increase in 4 and to decrease in 3 of seven subjects whom he studied. Lindhard concluded that the effect of irradiation was first and foremost a cutaneous dilatation which, if not counteracted in some way, would produce an increase in the minute volume flow of blood. Since, however, the regulating power may be different in different subjects, or in the same subject at different times, the end result, he believed, might be either an increase or a decrease and could not be foretold.

Our aim was to extend the observations on changes in blood pressure and pulse rate and, more particularly, to obtain data concerning the behavior of the cardiac output in normal dogs and in normal and hypertensive men after carbon arc irradiation.

PROCEDURE. In the dog experiments, the animals were trained to lie quietly on a padded table and preliminary determinations of blood pressure,

[1] The data on the dog are taken in part from the dissertation submitted by B. E. Pollock to the Graduate School of Tulane University in partial fulfillment of the requirements for the degree of Master of Science, June, 1933. Those on man are taken in part from the dissertation submitted by J. Raymond Johnson in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1934.

pulse rate, oxygen consumption, cardiac output and rectal temperature were made until the emotional response to such procedures had ceased, when normal basal levels were established. Blood pressure was measured by an adaptation of the auscultatory method (2). Oxygen consumption per minute was determined on a Benedict-Roth metabolimeter after the dog had rested quietly in a basal condition for an hour. Cardiac output was determined by the Fick principle, the Van Slyke-Neill manometric gas analyzer being used to measure the oxygen content of the blood samples.

The general plan in the human experiments was to establish a normal base line for oxygen consumption, cardiac output, blood pressure, pulse rate and, in some cases, hemoglobin content of the blood and then to follow these functions daily after single measured doses of carbon arc radiation. All of the observations were made with the subjects in a basal state, the tests being run in the forenoon from 12 to 16 hours after the last meal, after a one hour rest in a steamer chair or bed and without any strenuous mental or physical exercise since the day before. Cardiac output was determined by the acetylene method of Grollman (5), oxygen consumption with the Benedict-Roth metabolimeter and hemoglobin by direct measurement of the oxygen capacity by the manometric method of Van Slyke-Neill (6). Pulse rate and blood pressure were usually determined three or four times during the preliminary rest period as an index of the degree of relaxation, but only the last readings were recorded.

Two sources of radiant energy were used. In all of the dog and in some of the human experiments, a "Pan Ray" arc (25–28A, 50–60V) was used; in the rest of the work the source was an "Eveready" professional model (A-1) carbon arc. National "Sunshine" carbons were burned in all except a few experiments where "C" carbons were substituted. The total energy with its spectral distribution was determined for each exposure by the radiometric method described by Laurens (1). Massive doses were given in the dog experiments (40–60 min. at 75–100 cm.). In the human cases, the radiation intensity was varied (30–90 min. at 70–100 cm.) so as to produce a definite erythema, usually accompanied by some tenderness and subsequent peeling but without blistering. The erythema usually reached its greatest intensity by the end of 24 hours and then gradually disappeared during the course of the next 3 or 4 days. When there was more than one exposure of the same individual, the erythema was allowed to disappear and circulatory functions to return to approximately normal levels before the subject was again irradiated.

Results. Four experiments on 3 dogs were carried out with results as shown in figure 1. The minute output of the heart decreased in all, the amount varying between 20 and 45 per cent. The maximal change occurred in from 5 hours to 5 days after the irradiations with return to

normal by the fourth to ninth day. The output of the heart per beat also decreased markedly following irradiation, but its course in any instance does not closely parallel the minute output because of the rather wide fluctuations in pulse rate. The rate of oxygen consumption was increased during and for 1 to 3 hours after the actual exposure due, probably, to heating incident to the irradiation, but there was a rapid return to normal and no subsequent significant change in direction or amount. As a consequence of this general constancy of oxygen consumption accompanying a fall in the minute cardiac output, the oxygen utilization of the blood, i.e., the arterio-venous oxygen difference, increased between 17 and 52 per cent. Systolic pressure was uniformly lowered, the changes roughly paralleling those in cardiac output. The maximal depression ranged from

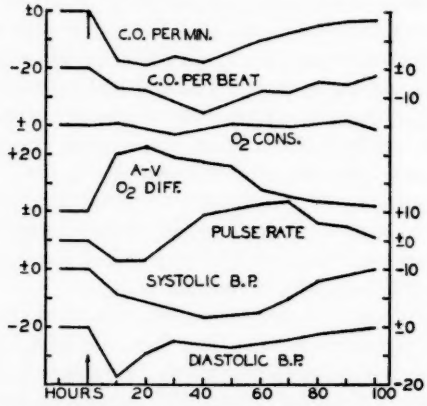

Fig. 1. Changes after single doses of carbon arc radiation. Normal dogs. The points represent the averages of all experiments expressed as per cent deviation from pre-irradiation values, except those for blood pressure which are given in millimeters of mercury. The arrows denote time of irradiation.

10 to 17 per cent maintained for from three to five days after the irradiations were discontinued. Diastolic pressure diminished more markedly than the systolic in the early period but returned to normal more quickly, the maximal depression ranging from 17 to 20 per cent. Pulse rates were variable with a tendency for the rate to fall during the first 24 hours with a subsequent rise above the normal level, reaching its height between 2 and 3 days after irradiation. Respiration and rectal temperature showed no significant changes except during or immediately after the actual irradiation.

In the work on humans, 52 exposures were made on 20 subjects. Of these 8 were males between the ages of 20 to 32 years and with blood pressures within the normal range (systolic = 94–112 mm., diastolic = 54–

78 mm.), while twelve were hypertensives. The latter were all ambulatory male patients from 21 to 67 years of age with pressures ranging from 128 to 191 mm. systolic and 74 to 121 mm. diastolic. One had a general arteriosclerosis, one a mild interstitial nephritis, and the rest were essential. Ten of the subjects were studied in the U. S. Marine Hospital in New Orleans where they were patients at the time, and the other two made visits to the laboratory of physiology for irradiations and tests.

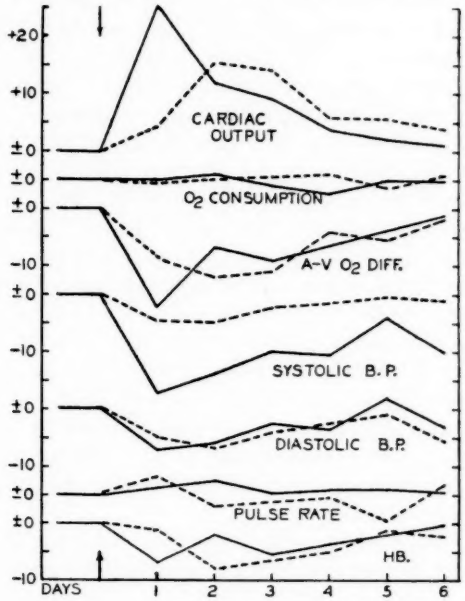

Fig. 2. Average daily changes after single doses of carbon arc radiation. Normal (- - -) and hypertensive (———) men. The points are determined by the arithmetic average of the changes occurring on each day, expressed as per cent deviation from pre-irradiation values, except those in blood pressure which are given in millimeters of mercury. The arrows denote time of irradiation.

The results are summarized in figure 2. The most striking effect in both groups was the increase in cardiac output which followed the irradiation. In the twenty experiments on the normal group there were only two in which the cardiac output did not show an appreciable rise (12 to 35 per cent) and these were both in the same subject. The average pre-irradiation value for the cardiac index in the twelve hypertensive subjects was 2.24, which is within the limits, 2.2 ± 0.2 liters per minute per square meter of body surface, given by Grollman (7) as the cardiac index for normal subjects, but the range of values for individual subjects was quite

wide: 1.64 to 2.79. These figures are in agreement with those of Starr, Collins and Wood (8) who found that some cases of hypertension showed outputs above and others below the normal values. They noted a very close correlation between the cardiac output and the size of the heart and suggested that the low output values found in certain cases represent an adjustment by the heart, possibly at the expense of other tissues, to prevent an increase in cardiac work and development of cardiac hypertrophy. Following irradiation there was an increase in cardiac output averaging 39 per cent in 21, a decrease averaging 23 per cent in 6, and no significant change in 5 experiments. The maximal change was generally evident on the first day after the irradiation in the hypertensive and on the second or third day in the normal group, with a return to normal in from 4 to 6 days. Oxygen consumption showed only insignificant changes in all the experiments so that the changes in the arterio-venous oxygen difference are roughly reciprocal to those of cardiac output. The results on oxygen consumption are in agreement with those of Eichelberger (9) and of Fries (10) and directly contradictory to those of Lindhard (4) who reported that oxygen consumption increased in approximately the same proportion as cardiac output, while oxygen utilization of the blood remained constant after carbon arc irradiation. Although the pulse rate showed fluctuations above and below pre-irradiation levels, they were in no case greater than normal variations during the control period. With this relative constancy of pulse rate, the output of the heart per beat necessarily increases in the same ratio as the minute output, thus suggesting an explanation for the finding of a fuller and stronger pulse after irradiation as reported by some investigators (11–13).

The blood pressure changes in the normal group are slight but with a definite tendency toward a lowering (6 mm. systolic and 8 mm. diastolic) lasting one to two days and with a slight increase in pulse pressure. These changes are of similar magnitude to those reported by Kimmerle (14) and Hasselbalch (15). In the hypertensives changes, of the systolic pressure particularly, are more marked and lasting, there being a definite fall in thirty-one of the thirty-two observations. The maximal depression of the systolic pressure ranged from 2 to 41 mm. Hg, that of the diastolic 2 to 20 mm. Since the depression in the systolic is relatively greater than in the diastolic, there is a definite decrease in the pulse pressure.

Hemoglobin estimations were made in 29 experiments to provide information regarding changes in blood volume. While the changes in hemoglobin do not follow quantitatively the blood volume changes, they may undoubtedly be used as an indirect criterion for determining the qualitative changes which occur, particularly when the hemoglobin values decrease. On the other hand, when the values show no change, or a slight increase, the addition of extra hemoglobin to the circulating blood may have over-

shadowed the tendency to dilution which would be produced by an increase in fluid content. The results of these determinations show that in both groups there is a predominant tendency for blood volume to increase after irradiation, since in 17 cases the hemoglobin content of the blood decreased from 5 to 11 per cent, in 10 cases there was no significant change and in only 2 experiments was there any indication of an increase. That carbon arc irradiation of the dog results in an increase in blood volume has been shown in previous work (3).

Discussion. The comparative ease with which the blood pressure of dogs may be lowered by carbon arc irradiation and the concomitant decrease in cardiac output, as found in these experiments, supports the previous work from this laboratory (2). The reasons for the difference in the cardiac output response in the dog and the human are, at present, not clear. If, as Lewis (16), Ellinger (17) and others believe, there is a production of some histamine-like substance in the skin by ultraviolet radiation, it may be that the dog is more sensitive to its presence than is man. The resulting condition after irradiation may approach that of shock, in which, because of the capillary dilatation and paralysis, the venous return and secondarily the cardiac output and blood pressure would be diminished.

The question as to whether or not the increase in cardiac output in normal individuals after an irradiation is a compensatory reaction cannot be answered now. The decreased blood pressure may be a direct effect of vasodilatation and the increase in cardiac output and blood volume compensatory reactions to prevent the fall in blood pressure from being too great. On the other hand, the changes in cardiac output and blood volume may be purely mechanical effects resulting from the lowered peripheral resistance and diminished hydrostatic pressure. If hypertension is a compensatory reaction (18), then the increased sensitivity of the hypertensive group may be regarded as a further attempt on the part of the circulatory system to maintain a compensated state after the vasodilatation produced by the irradiation. The increase in the cardiac output in some subjects after irradiation, and the absence of an increase in others, suggests further the possibility that this ability to compensate may be better developed in some individuals than in others. If, on the other hand, the increased cardiac output is a mechanical result of lowered peripheral resistance, then it should occur in every instance where there is a fall in blood pressure. The same holds true for the blood volume; if the changes are purely mechanical, then there should be an increase every time the blood pressure is lowered. But, like the cardiac output, there are several instances, corresponding in practically every case with a diminished cardiac output, in which the blood volume decreases also. This makes it seem quite likely that the changes in these functions are compensatory, but that there are varying degrees of ability to produce this compensation in

hypertensives. Where it is least developed the cardiac output and circulating blood volume may actually decrease, due to the production of a near shock-like condition in which there is a varying degree of capillary stasis. In normals this ability to compensate, although not necessarily any greater, is of more frequent occurrence, so that in practically every case there is an increase in the cardiac output and blood volume.

That hypertensives are more sensitive to radiation than normals is suggested by the work of Ellinger (19). Using erythema production on the arm as his criterion, he found the sensitivity in a group of 12 hypertensives between the ages of 20 and 50 years to be nearly 1.5 times as great as in normals of the same age. In 23 patients over 50 years of age it was 2.5 times as great as the normal for the same age. Due to the small size and heterogeneity of the group, Ellinger does not draw any definite conclusions from his data. The evidence is strengthened, however, by his study of a group of 13 individuals with low blood pressure in which the sensitivity was only half of what it is in normals. Ellinger suggests that the increase in sensitivity, which he has demonstrated in other conditions as well, may be tied up in some way with improved circulation in the skin, in turn due to increased thyroid activity.

The vasodilatation produced by irradiation is considered by some to be limited to the skin area while others believe it extends to the internal vessels as well. If the reaction is only superficial, the increased blood volume speaks against any marked degree of compensatory vasoconstriction elsewhere with merely a redistribution of blood. Often, particularly in the normal subjects, the greatest increase in cardiac output occurred on the second or third or even the fourth day following the exposure, while the erythema always reached its height during the first 24 hours. This would suggest a gradual spread of the vasodilatation, which might be best explained by assuming that histamine or some H-substance is produced in the skin and circulated throughout the whole body. Much more information is needed as to the action of histamine and H-substances in the dog and in normal and hypertensive individuals before these questions can be satisfactorily answered. Weiss, Ellis and Robb (20) studied the effects of histamine following continuous intravenous injection in human subjects both with normal and elevated pressures and found that in doses of from 0.02 to 0.04 mgm. per minute the blood pressure either remained unaltered or underwent very slight, transient and inconstant fluctuations both upward and downward. They continued their injections of histamine over a relatively short period of time as compared with a 2 or 3 day period, and whether or not a continuous production of even smaller amounts over a prolonged period such as this would have a greater effect is not known.

It is a pleasure to record our thanks to Major T. B. H. Anderson, Super-

intendent of the U. S. Marine Hospital in New Orleans, and to Major W. I. Hollingsworth, the Commanding Officer in charge, for permitting us to work in the hospital.

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SUMMARY

1. The changes in cardiac output, blood pressure, oxygen consumption, pulse rate and hemoglobin content have been studied in a group of dogs, and in normal and hypertensive men after single, erythema producing, doses of carbon arc radiation.

2. The outstanding effect in the dog is a diminution in both blood pressure and cardiac output.

3. In normal men the blood pressure shows a slight lowering (6 mm. systolic, 8 mm. diastolic) lasting 1 to 2 days accompanied by an increase in cardiac output averaging 21 per cent. The highest value for cardiac output is reached on the 2nd or 3rd day after irradiation with a return to normal by the 5th or 6th day. Only insignificant changes are found in oxygen consumption.

4. In hypertensives there is a consistent and more marked lowering particularly of the systolic blood pressure, the average drop being 17 mm. for the systolic and 7 mm. for diastolic pressure. The cardiac output increased in twenty-one instances by an average of 39 per cent, decreased in six by an average of 23 per cent, and showed no significant change in five. The changes in oxygen consumption and pulse rate are small and inconstant. Hemoglobin changes indicate that, as a rule, whenever the cardiac output increases there is a corresponding increase in the blood volume and when it decreases there is possibly a diminution in blood volume.

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THE EFFECT OF FAT ON THE pH OF THE CONTENTS OF THE DUODENUM

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Our interest in the present problem was aroused by finding in a recent edition of a standard text the statement that fats in the diet probably increase the acidity of the intestinal contents. A few preliminary experiments suggested quite the opposite and we determined to investigate the matter more fully.

There is an extensive literature dealing with the pH of the intestinal contents under a variety of conditions. Although some studies have dealt with the influence of different diets administered over comparatively long periods of time, few have been undertaken to determine the influence of the type of food undergoing digestion at the time the observations were made. We know of none in which this has been the primary object of the investigation.

McClure, Montague and Campbell (1924) studied the intestinal contents in man after meals consisting of 25 grams of either edestin, olive oil, arrowroot starch or a mixture of the three (8.33 grams of each). They found the intestinal contents acid after the protein or the mixed meal and alkaline after the fat or the carbohydrate meal. Their pH values are, on the whole, higher than have since been reported, probably because of the small amounts of food given.

Recently, Mann and Bollman (1935) and their collaborators (Stephens, 1935; Imes, 1935; McRoberts, 1935; Hoerner, 1935a, 1935b) have reported a study of certain properties of the duodenal contents, including pH, during digestion of a variety of food substances. Our study was nearing completion when their work was published. We found that we had confirmed many of their results under similar experimental conditions. However, their work fails in certain essentials to supply the data required for the solution of our problem.

METHOD. Observations were made on 4 dogs, all females weighing between 20 and 30 kilos. Three of them were provided with cannulated gastric and duodenal fistulas as previously described (Thomas, Crider and Mogan, 1934). In the fourth animal the duodenum was made accessible by bringing it out to a lateral abdominal incision and suturing a

portion of the serous surface to the skin so that after healing a small area of duodenal wall remained exposed. This animal was prepared for the purpose of revealing any possible influence of the fistulas in the other animals. No difference in results attributable to the fistulas was observed.

Studies were made after various test meals which will be described later. Samples of the duodenal contents were collected from a point estimated to be 6 to 8 inches from the pylorus, at 20 to 30 minute (occasionally longer) intervals over a period of 5 to 7 hours. The first sample was taken before feeding if possible; if not, as soon after as it could be obtained.

The pH determinations were made at room temperature on a saline dialysate of the duodenal contents by means of a Hastings-Dubosc q colorimeter. The material was dialysed through cellophane in a chamber designed to prevent loss of carbon dioxide. Every important detail of the method, including preparation of the indicator solutions, the indicator constants used, the time required for dialysis equilibrium, and finally the whole procedure in the course of routine determinations, was checked electrometrically. The final electrometric-colorimetric difference rarely amounted to 0.2 pH unit. A few experiments are recorded in which colorimetric determinations were made directly on centrifuged samples; in these the error is probably somewhat greater.

Results. *Raw lean meat.* In order to establish a basis for comparison 12 experiments were performed on 4 dogs in which the test meal consisted of raw beef from which all gross fat had been removed. The standard meal was 400 grams but in one instance each 300, 500 and 600 grams were fed. The results are shown graphically in curve A. The pH of the duodenal contents decreased rapidly after feeding and by the end of the first hour had attained the level characteristic of the digesting state. This level was slightly higher in the latter half of the experimental period. Except in occasional instances, which became more numerous toward the end of the period of observation, the pH values found after the first hour ranged between 3.8 and 4.6. The approximate average of these results is indicated on curves B, C and D by triangles and a broken line.

The results agree with those obtained by Mann and Bollman and their collaborators with 100 or 200 grams of horse meat as a test meal, except for a narrower range of variation in our experiments. Comparing our results with theirs it is evident, as they found, that larger meals cause a more prolonged increase in duodenal acidity.

Fat alone. In 3 experiments 2 animals were given 200 grams of raw beef suet. A third animal refused to eat the suet and was fed 115 grams of butter instead. In none of these was there a consistent increase in acidity after feeding. Except in a few instances, which were more numerous after the butter meal, the pH of the samples ranged throughout between 6.0 and 7.0. Most of the values below pH 6.0 were obtained after

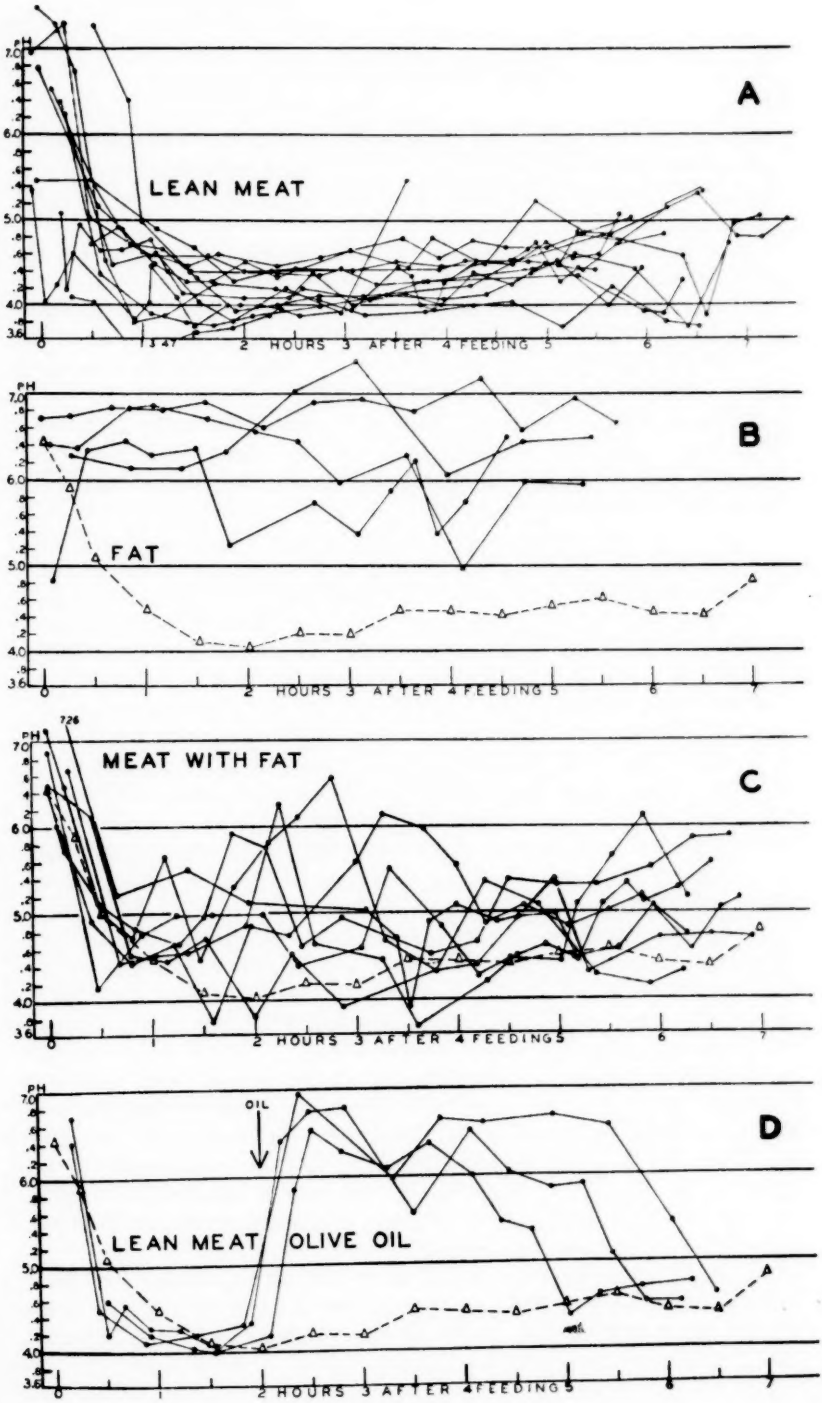
A
LEAN MEAT
B
FAT
C
MEAT WITH FAT
D
OIL
LEAN MEAT OLIVE OIL
HOURS AFTER FEEDING

Fig. 1

butter and those above pH 7.0 after the suet meals. The results are represented graphically in curve B. They agree with those reported by Mann, Bollman and their collaborators as following a meal of lard and crackers or of fresh horse fat.

Fat with meat. In 7 experiments on 4 animals the meal consisted of 100 grams of raw beef suet in addition to 400 grams of raw lean meat. The results during the first hour did not differ materially from those obtained in the same period with meat. Thereafter the pH of the samples fluctuated over a wide range and was, on the whole, higher than after the meat alone. The results are illustrated in curve C.

The gross appearance of the samples gave some clue to the significance of the pH variations. Ordinarily, free fat was not conspicuous in the samples obtained during the first hour. Later there was a fairly consistent relation between the amount of fat in the samples and the pH variations, the pH tending to increase *after* a sample containing more than the usual amount of fat had been obtained. Frequently very acid samples contained large amounts of fat. It is perhaps significant that such samples could be cleared by centrifuging and gave no evidence of the presence of emulsified fat. Some such samples contained no bile and after centrifuging were as clear and colorless as water. It was noted also that the less acid the sample, the more opaque it was likely to be, presumably due to emulsified fat.

We think that even greater fluctuations than we have recorded would have been observed in these experiments had we been able to collect our samples in a shorter time. From 5 to 20 minutes were required to collect the relatively large sample needed for dialysis. It is evident that the pH of any sample tends to approximate the mean pH of the duodenal contents during the collection time.

Oil given in the course of meat digestion. In 3 experiments on 3 dogs, two hours after the standard meat meal was fed, the animal was offered olive oil. Two of the animals swallowed the oil willingly, taking 50 cc. each. The third animal refused the oil and it was administered through the gastric cannula; this animal received 100 cc. The results were similar in all except that the larger amount of oil produced a more prolonged response. The pH of the duodenal contents rose sharply during the first half hour after the oil was given, from the level characteristic of meat digestion (4.0 to 4.4 in these experiments) to that characteristic of fat digestion, i.e. between 6.0 and 7.0. It remained at this level for 2 hours after 50 cc. of oil and for nearly 4 hours in the experiment with 100 cc. The results are illustrated in curve D.

Incidental observations: bread; egg white and yolk; conditions not related to the type of food. In three experiments the animals were offered white baker's bread and allowed to eat all they would take. They ate 260,

190, and 110 grams respectively. The results were slightly different from those reported by Mann and Bollman and their co-workers, using syrup and milk. They found little difference between the effect of their carbohydrate meal and of fat on duodenal pH. In our experiments the contents were slightly but consistently more acid after bread than after fat. The range was from pH 5.0 to pH 6.8 but most of the samples were below pH 6.0.

Two animals were fed 6 boiled eggs each. The whites were given first and several hours later, the yolks. The duodenal contents were less acid after egg white than after meat, the range being approximately the same as after bread. Feeding the yolks caused no further change.

In a few experiments the animal was given the standard meat meal when the stomach was known to contain bone chips, hair or food residues. In such instances the characteristic degree of acidity failed to develop in the contents of the duodenum. All such experiments were discarded; they are, nevertheless, instructive in that they show the influence on duodenal pH of factors other than the type of food. We think that in these instances either the condition of the stomach interfered with appetite and, consequently, with gastric secretion, or the presence of indigestible material retarded gastric emptying.

Discussion. The results furnish an answer to the question raised in the introductory sentences; fat tends not to increase but definitely to diminish the acidity of the contents of the duodenum. However, their greatest value probably lies in the light they shed on the significance of the inhibitory action of fat on gastric secretion and motility. Although this phenomenon has been studied extensively, very little has been learned about its usefulness in digestion.

There are many reasons for thinking that efficient fat digestion requires a less acid medium than is frequently found in the duodenum. Since digesting fat tends locally to increase acidity, it is evident that the observed decrease in acidity must be due to more remote physiological effects. The work of Mann and Bollman and their collaborators provides convincing evidence that the dominant factor among the several that determine the pH of the duodenal contents during digestion is the amount of acid entering from the stomach. The inhibitory influence of fat on gastric secretion and motility is therefore probably mainly responsible for its effect on duodenal pH. If this is true, the mechanism that enables fat to inhibit the gastric functions may be regarded as an adaptation serving to adjust the acidity of the duodenal contents to the requirements of fat digestion.

The results with fat are evidently specific for this substance since they were obtained with a variety of fatty materials which were of different physical and chemical constitution. The evidence for a specific influence of carbohydrate on duodenal pH is suggestive but inconclusive. In the

case of protein, the available data point to the absence of a specific influence. The difference in results with egg white as compared to meat suggests that the effect of the latter is not necessarily due to its protein content. Palatability and the presence of gastric secretagogues are possible factors in the effect of meat.

SUMMARY. 1. Following a meal of fat the acidity of the duodenal contents is increased little if any over the fasting level.

2. After a meal of solid fat mixed with meat the acidity of the duodenal contents is less than after meat alone. Marked fluctuations in acidity occur which are associated with the intermittent appearance of gross fat in the duodenal contents.

3. The administration of oil in the course of meat digestion causes a prompt decrease in the acidity of the duodenal contents.

CONCLUSION

Fat modifies gastrointestinal activity in such a manner as to render the duodenal contents neutral or only slightly acid. Presumably this is due to its inhibitory effect on gastric secretion and motility. This mechanism may be of importance in providing a suitable medium for fat digestion.

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THE PHASIC AND MINUTE CORONARY FLOW DURING ACUTE EXPERIMENTAL HYPERTENSION

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The phasic and minute coronary blood flow under various dynamic conditions is still in dispute. While it is concluded by Starling and associates (1914, 1922) and by Anrep and associates (1928, 1929, 1931), that coronary flow occurs entirely during diastole and hence is largely governed by the aortic pressure during diastole, Hochrein (1931), Rein (1931) believe that the major part of the flow is limited to systole and that heart rate and output have a greater effect on minute flow than mean blood pressure. Recent evidence from this laboratory (1935) indicates that in a single heart beat there is a definite systolic coronary blood flow. The method used consisted essentially in reconstructing (from original records) a differential pressure curve of the central and peripheral pressure variations in a coronary ramus. The height of the curve at any point gave velocity of flow and the area under any portion gave volume flow (cf. also curve D, fig. 2).

Utilizing the areas under such differential pressure curves it should be possible to make theoretical deductions as to the effects which separate variables acting one at a time have on the phasic distribution of volume flow per beat and on minute flow (i.e., the product of heart rate and flow per beat). For example, an increased heart rate should decrease the flow per cycle and per minute chiefly because the time that the heart is in systole increases while the time that it is in diastole (in which there is a greater blood flow) generally decreases. Unfortunately such an analysis is of no physiological importance since in the intact circulation the form, duration, time relations and magnitude of the peripheral coronary pressure (P.C.P.) variations as well as those of the central aortic pressure variations, would be expected to change.

From such considerations it is apparent that the question of the coronary circulation under different conditions must be put to the test of actual experimentation capable of giving information, especially on the peripheral coronary diastolic and systolic resistance. Therefore an attempt was made to study resultant changes in coronary flow occasioned primarily by

an increased arterial resistance and an effort was also made to analyze the relative importance of secondary changes in heart rate and systolic discharge which accompanied it. Such an approach seemed particularly useful since the results obtained by previous investigators apply more particularly to the dynamic changes which result when rate and output increase while the left ventricle discharges against approximately normal resistance. In other words, while changes in coronary flow, pertaining to conditions such as are realized during muscular exertion, have been repeatedly studied, the alterations that exist during states of hypertension have been studied relatively less.

PROCEDURE. All experiments were performed on anesthetized dogs with open chest and intact vagi. A main left coronary ramus and a suitable side branch were isolated. The central aortic pressure and the central and peripheral coronary pressure variations together with the maximum systolic coronary resistance were recorded by the method and apparatus previously described (1935). After a series of normal records, further curves were recorded during gradual elevation of the aortic pressure.

Elevation of arterial pressure was produced in two ways: 1, by compression of the thoracic aorta just above the diaphragm which produced changes of coronary flow depending upon natural physiological reactions, and 2, by slow intravenous infusion of epinephrine or synephrine which might additionally affect coronary flow through direct effects on coronary vessels and through further augmentation of ventricular contraction. From these curves and data reconstructions were made depicting the variations in velocity and volume flow in a single heart cycle (fig. 2). The product of volume flow per beat and heart rate gave the minute flow expressed as square millimeters of area under the velocity curves, D, of figure 2. The effects of different variables upon coronary flow, such as heart rate and output, could generally be separated by comparing events at different times during the course of an experiment. However, supplementary experiments were also performed in which either the heart rate was altered by rhythmic stimulation of the right auricle (after first clamping the S.A. node) or the output was increased by rapid saline infusion while the heart rate was kept constant and the blood pressure was relatively the same.

RESULTS. The changes in aortic pressure, heart rate and coronary flow during hypertension are illustrated by a graph of a typical experiment as in figure 1. A number of reconstructions of volume and velocity flow, which supply in part the data for the systolic and total minute flows in figure 1, are illustrated in figure 2. To prevent confusion the letters referring to the charts in figure 2 correspond to those inscribed on the flow curves in figure 1 and only the aortic pressures (A), the peripheral coronary pressure curves (C) raised to their proper ordinate values and the differential flow curves (D) are reproduced in figure 2.

In figure 1, the control aortic blood pressure was 113/78 mm. Hg and the heart rate was 146 per minute. During aortic compression by graded increments the pressure rose to 178/133 mm. Hg at G while the heart rate decreased to 135 per minute. During decompression the pressure was permitted to fall to 73/43 mm. Hg at K, and the heart rate dropped to 65 per minute. With this slow heart rate prevailing the aorta was again

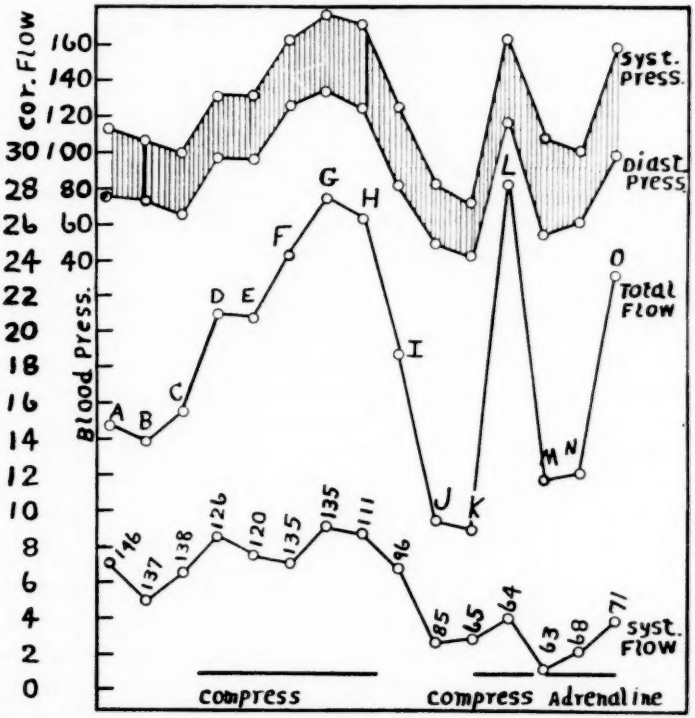

Fig. 1. Graph showing effect of acute hypertension on phasic and total blood flow per minute in ramus descendens anterior. Numbers on systolic flow curve = heart rate. Ordinate values = millimeter mercury pressure and flow per minute $\times 10^3$ square millimeter area. (Heart rate 65 at K should read 69.)

compressed so that the pressure rose to 163/117 mm. Hg at L. After a second decompression, epinephrine was slowly infused as a result of which the pressure rose in successive records to 160/100 mm. Hg with essentially the same heart rate, 71 per minute.

As shown in figure 1, the total minute coronary flow, the systolic minute flow and diastolic minute flow (i.e., the difference between the two flow curves) all rise and fall with the aortic pressures. It is also apparent that

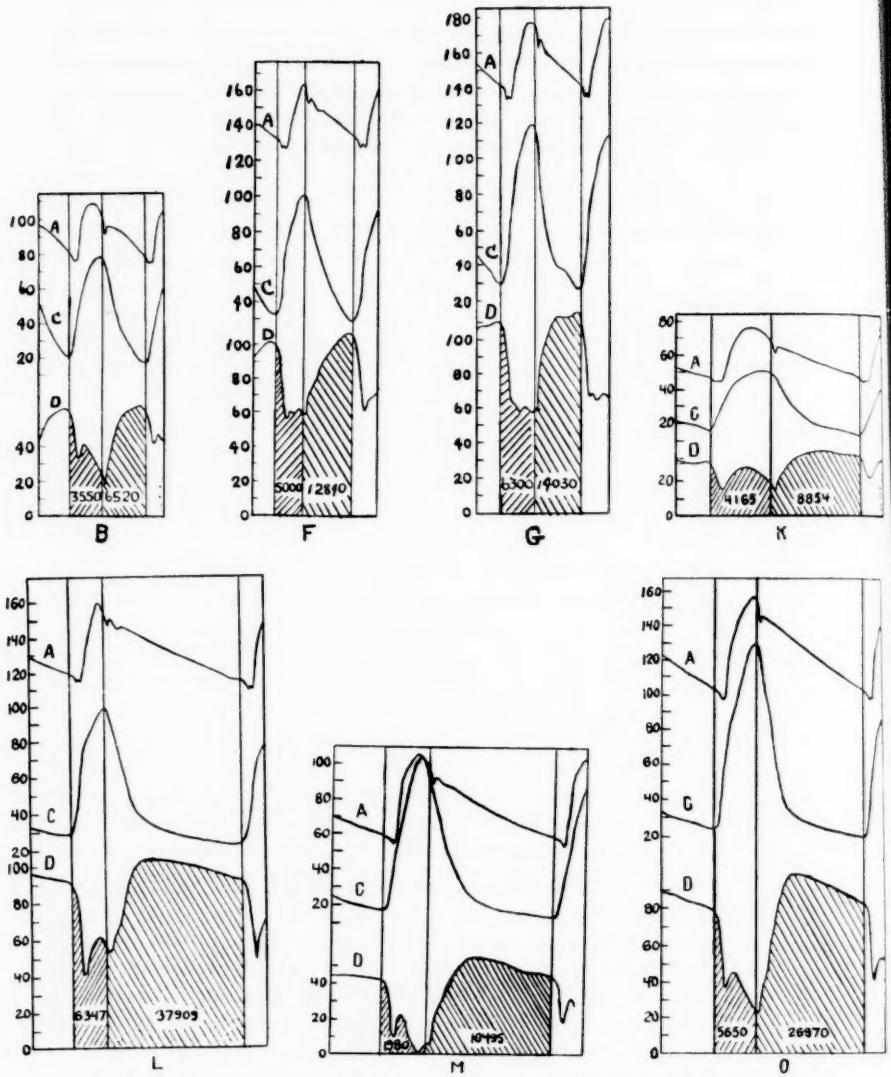

Fig. 2. Charts depicting phasic distribution of blood during a single heart cycle in ramus descendens anterior. A = aortic pressure curve, C = peripheral coronary pressure curve raised to its proper ordinate value. D = velocity curve. Shaded areas = flow in systole and diastole expressed as square millimeter of area $\times 10^5$. Letter for each chart refers to same point on flow curve, figure 1. Ordinate values = millimeter mercury pressure.

the diastolic flow changes more profoundly than the systolic. Such curves, however, give no detailed insight into the mechanisms involved. The graphs and areas of figure 2 may be used to yield information on the fundamental causes of these alterations in flow.

In figure 2, chart B as compared with F, the blood pressure increases from 107/74 to 163/126 mm. Hg through aortic compression, while the heart rate is constant at 136 per minute and the pulse pressure (and presumably systolic discharge) rises only slightly (33 to 37 mm. Hg). A comparison of total shaded areas of the lower graph shows that the flow per cycle and per minute (since the heart rate is constant) increases 78 per cent. Of this additional blood flow 20 per cent occurs in systole and 80 per cent in diastole. The reasons for this become clear upon inspection of the pressure curves. The systolic flow is augmented because the effect of a decrease in systolic duration is more than compensated by the increased systolic velocity of flow. The latter is occasioned by the fact that the systolic coronary resistance (C), although elevated considerably in chart F (100 mm. Hg) as compared with chart B (80 mm. Hg) rises much less than the aortic pressure. In fact the ratio of the maximum coronary systolic resistance to the aortic systolic pressure decreases from 73 to 59 per cent. This phenomenon of a relatively smaller increase in systolic resistance to flow upon increasing the peripheral resistance is a characteristic finding in a large number of experiments. Such results are diametrically opposed to those of Anrep, Davis and Volhard (1931) who affirm that there is a measurable systolic flow only when the heart is in a hypodynamic condition. In this instance with an aortic pressure of 163 mm. Hg there can be no doubt that the left ventricle is contracting vigorously.

The diastolic flow is augmented in part by a slightly longer diastole at the higher pressure in F, but chiefly because the aortic pressure is much greater throughout diastole, while the pressure is only slightly raised (22 to 33 mm. Hg) in the peripheral coronary vessels.

Comparison of charts K and L, figure 2, shows the same effect of increasing aortic pressure when the heart rate is unchanged but slower than in charts B, F, that is, the minute coronary flow increases chiefly because of an augmented diastolic flow.

The contrasting effects of hypertension created chemically and mechanically are set forth in the charts L, M, O, figure 2, in which the heart rate is essentially the same. After record L, the aorta was gradually decompressed. Then a small amount of epinephrine was infused into the jugular vein. The record from which M was reconstructed was then taken. The most outstanding effect is the rise of the coronary systolic resistance to a value equal to the aortic systolic when the blood pressure is 107/55 mm. Hg and the heart rate is 63 per minute.

Despite this, however, the systolic flow per beat is only reduced to 12 per cent of the cyclic flow because the contour and time relations of the aortic and P.C.P. curves are not identical. The systolic flow is less and the systolic coronary pressure resisting flow is greater than we have ever encountered in any other experiments with comparable heart rates and aortic pressures and in which epinephrine had not been administered.

After additional infusion of epinephrine and also synephrine through the coronary cannula, chart O was reconstructed from the records taken. This displays approximately the same heart rate (71 versus 64 per min.) and a degree of hypertension (159/99 versus 163/117 mm. Hg) similar to that in L. Comparison shows that the systolic coronary resistance in O (129 mm. Hg) is much higher than in L (100 mm. Hg). As regards diastole the flow decreases as compared with L, for the length of diastole and flow velocity are both less, despite a slightly lower coronary diastolic pressure (24 mm. Hg in O as compared to 28 mm. Hg in L).

Whatever the ultimate mechanisms may be, the creation of high blood pressure with epinephrine increases the systolic resistance to flow in the coronary arteries and, when the aortic pressure is not too high, completely stops coronary flow for a fraction of systole. Such results confirm the observations of Anrep, Davis and Volhard (1931) that momentary cessation of systolic flow can occur when the beat of the ventricle is very vigorous but it must be emphasized that these effects do not occur in natural responses of the heart to increased resistance and only supervene when the left ventricle is intensely stimulated by large doses of epinephrine. Moreover even in such extreme conditions the systolic volume flow is by no means zero.

Heart rate as a factor in determining coronary flow. Considerable difficulty was experienced in finding records in which the possible effects of changes in heart rate during aortic compression could be separately determined. However, in figure 2, charts G, L, other variables are reasonably constant. The heart rate decreases from 135 in chart G to 64 per minute in chart L (presumably through reflex vagal action) while the blood pressure falls slightly from 178/134 to 163/117 mm. Hg and the pulse pressure remains essentially unchanged at 44 and 46 mm. Hg. At the slower rate in L the systolic volume flow per beat is unchanged while the minute systolic flow is reduced 52 per cent. The latter is brought about by the fewer number of systoles per minute.

The diastolic flow per beat and per minute is augmented primarily because the duration of diastole increases and secondarily because the diastolic coronary pressure decreases slightly.

As a resultant of an unchanged systolic and increased diastolic flow per beat upon halving the heart rate, the minute flow changes only slightly (27.44 to 28.33). Since these experiments are complicated somewhat by blood pressure changes, further experiments were done in which the

effects of change in heart rate could be better assessed. Reconstructions from such an experiment in which the heart rate was increased from 68 to 116 per minute by auricular stimulation are presented in figure 3 A, B. At aortic pressures of 78/49 and 76/55 mm. Hg the results as regards flow are similar to those in charts G, L, figure 2. They yield the additional information that at the faster heart rate the systolic volume flow per beat is decreased and the velocity of flow may equal zero late in systole. This heightening of coronary systolic resistance has been observed in most records. Whether this will be found to be the rule in a larger series of experiments remains to be determined.

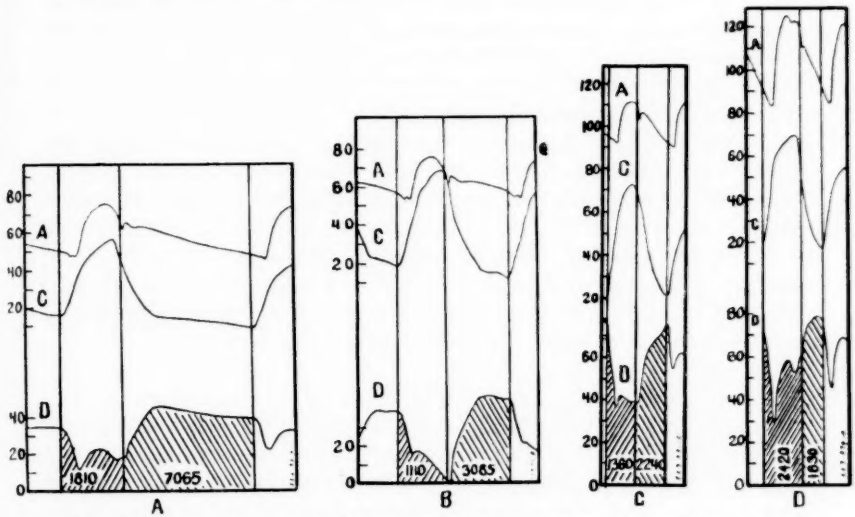

Fig. 3. Charts representing changes in coronary flow per heart beat in ramus descendens anterior upon increasing heart rate and systolic discharge. Chart A = control. B = plus heart rate. C = control. D = increased systolic discharge. Other letters same as in figure 2.

Such calculations of flow indicating a slight reduction of minute flow when the heart is mechanically accelerated accord with those of Anrep and Hausler (1929), but they are contrary to those of Rein (1931) and Hochrein (1931).

Pulse pressure as a factor in determining coronary flow. Charts F and L, figure 2, are taken for comparison because in addition to exhibiting a heart rate decrease from 135 to 64 per minute, the pulse pressure increases from 37 to 46 mm. Hg while the blood pressure is essentially unchanged. Since the effect of this same change in heart rate has already been analyzed (fig. 2, G, L,) these charts allow us to study the effects of pulse pressure.

During systole the volume flow in chart F with the smaller pulse pressure is considerably less than in chart L.

During diastole in chart F, the flow per beat is decreased by the algebraic sum of a slightly higher aortic diastolic pressure which increases flow, and the elevation of coronary diastolic from 28 to 33 mm. Hg plus a shorter diastole, both of which retard flow.

Comparison of the minute flow in charts F, G and L indicates that the flow in F with the smaller pulse pressure is somewhat less than that in charts G or in L (24.26 as compared with 27.45 and 28.33).

Such comparisons indicate that pulse pressure probably plays but a small rôle in determining coronary flow. Since the argument might be advanced that the change in pulse pressure was too small to be effective, additional experiments were performed in which the heart was mechanically driven and the systolic discharge of the left ventricle increased by the infusion of isotonic sodium chloride solution at body temperature into the jugular vein. Figure 3, charts C, D, shows a typical result. While the pulse pressure increases 50 per cent and the ratio of coronary systolic pressure to aortic systolic decreases from 66 to 56 per cent, the volume flow per cycle is increased 18 per cent. However, the systolic percentage of total flow increases from 38 to 57 per cent. This large change in phasic distribution of coronary flow coincident with a moderate increase in total volume flow is chiefly occasioned by the lower systolic coronary resistance and the marked lengthening of systole at the expense of diastole.

SUMMARY

The results presented elucidate the causes of the augmentation of coronary blood flow when the aortic pressure is elevated by increasing the peripheral resistance either mechanically or chemically. The essential factors which affect the distribution of flow between systole and diastole per beat are the relative magnitude, contour, time relations and duration of the systolic and diastolic phases of the aortic and P.C.P. curves. The sum of the pressure differences involving all these factors gives an index of the flow per beat and the product of cyclic flow and heart rate gives an index of the minute flow.

When all these effects are integrated during hypertension the picture becomes somewhat complicated. Tentatively our concept is that as the aorta is compressed and the blood pressure and pulse pressure rise, the heart rate slows, the coronary systolic resistance decreases relative to the aortic systolic, thus permitting an actually greater inflow per beat during ventricular contraction, although the systolic flow relative to diastolic is decreased. However the minute systolic flow increases only moderately since as the heart rate slows the time occupied by systole is materially reduced. During diastole at the high pressures the diastolic interval

lengthens and the diastolic coronary pressure increases only slightly (a resultant of the slower heart rate tending to decrease it and the higher blood pressure tending to raise it) with the net result that the diastolic flow per cycle and per minute is tremendously augmented. Quantitatively, although the flow in both phases of the cardiac cycle is greater, the augmentation of flow is chiefly diastolic.

When the hypertension is produced by epinephrine the same alterations of flow occur, except that the coronary systolic resistance may approximate aortic systolic, if the latter is not too high, with the result that systolic flow per beat and per minute is reduced to a small value.

Finally the conclusion is reached that the dominant factor increasing the myocardial blood supply in acute experimental hypertension is a combination of mechanical changes of which the most important is the aortic head of pressure throughout the cycle together with the relative increase in the time per minute occupied by diastole. Aiding this are additional mechanisms: the relative decrease of systolic coronary resistance at high aortic pressures and pulse pressures; the slightly decreased coronary diastolic resistance at the slower heart rates, and finally, slight changes in the form of the P.C.P. curve.

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THE EFFECT OF THYROXIN ON THE TISSUE METABOLISM OF EXCISED LIMULUS HEART

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In a previous article (1) experiments were described showing that the metabolism of the excised intact frog heart was increased after 10 to 15 hours of perfusion with thyroxin-containing Ringer's solution. These experiments did not show whether the increased metabolism was due to increased rate of beat (2, 3, 4) so recourse was had to the Limulus heart, which was better adapted for this purpose.

The Limulus heart consists of a long sack-like structure whose beating depends on the presence of the ganglion in the median nerve. If the median nerve is intact, the heart beats; if it is dissected out, the heart ceases to beat. If the heart is cut into sections without otherwise injuring the median nerve, these segments will also beat. With the Limulus heart, therefore, it was possible to determine not only the effect of thyroxin on the non-working heart but also whether the presence of the ganglion was necessary for the thyroxin effect.

In order to determine these effects, measurements were made by the Warburg differential method (5) of the respiration of segments of Limulus heart, both with and without its median nerve and with and without the addition of thyroxin. Twenty series of experiments were carried out. Five were run to determine the respiration of the heart with ganglion and 5 without ganglion—both groups being with the addition of thyroxin and with measurements made at the end of 1, 5, 10, 15 and 20 hour intervals after the initial determination. There were also 10 corresponding series without thyroxin, which served as controls. Only two determinations were made on each heart, an initial and a final one. In every case the initial determination was made in sea water without thyroxin. Between the initial and final determinations the hearts were kept in sea water at about 2°, with thyroxin for the first 10 series, without for the controls. For the final determination the hearts were thoroughly rinsed and placed in fresh sea water either with or without thyroxin, corresponding to their previous treatment. All determinations were made with the liquid media in equilibrium with air or oxygen.

The results of these experiments are shown in table 1. This table shows that in every case without thyroxin the oxygen consumption of the Limulus heart, whether with or without its ganglion, fell off considerably after the initial determination. In every case with thyroxin, the oxygen consumption of the Limulus heart, whether with or without its ganglion, showed a similar falling off up to 10 hours after the initial determination —at which time the falling off became less, suggesting that the effect of thyroxin was beginning to be exerted then. After 15 hours with thyroxin there was a marked increase both when the ganglion was present and when it was not. Similar increases (1) had been previously observed when thyroxin was added to the excised intact frog heart.

TABLE 1

Effect of thyroxin on metabolism of excised Limulus heart

TIME AFTER THYROXIN	CHANGE IN MEAN O_2 CONSUMPTION COMPARED WITH INITIAL VALUES			
	No. of expts.	Without thyroxin	No. of expts.	With thyroxin
		With ganglion		
hours		*per cent*		*per cent*
1	10	−21	8	−22
5	6	−6	8	−15
10	6	−17	4	−9
15	8	−30	6	+56
20	10	−20	18	+46
		Without ganglion		
1	6	−36	4	−30
5	8	−14	4	−17
10	5	−15	8	−3
15	8	−26	12	+34
20	10	−34	10	+57

These results seem to warrant the following conclusions:

1. That under suitable conditions thyroxin in vitro increased the metabolism of well-surviving segments of excised Limulus heart.

2. That the increased metabolism observed was not due to increased work by the heart.

3. That the effect of thyroxin was probably not exerted through the nerves but directly on the cells of the heart muscle.

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ON THE USE OF LAPICQUE'S FACTOR FOR CONVERTING VOLTAGE-CAPACITY TO STRENGTH-DURATION CURVES

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It is assumed, sometimes, that the direct current strength-duration curve of electrical excitation can be obtained from the corresponding voltage-capacity curve by means of Lapicque's (1926) factor, 0.37 cr. That is, it is assumed that for each point of the condenser curve, if the capacity in farads is multiplied by the resistance in ohms and by 0.37, the resulting time in seconds will be the duration which would be required for a direct current stimulus of the same voltage. Actually, however, this factor, 0.37, is an average ratio, experimentally determined, of the direct current chronaxie to the *cr* of the condenser stimulus whose voltage is 2 rheobases. Therefore, in origin, it has nothing to do with the relation of the whole of the two curves of excitation to each other but is concerned only with the practical matter of getting a probable value for chronaxie from the corresponding condenser datum.

It can be seen easily, on consideration, that in order to obtain one curve from another it is necessary that all the parameters of the one be given by the other. For example, in the writer's equations (1932),

$$\log \frac{V}{V - R} = kt + C \qquad (1),$$

for direct current stimuli and

$$\frac{V}{R} = (crk)^{\frac{1}{crk-1}} \qquad (2),$$

for condenser stimuli (where V and R are the stimulating voltages and rheobases, respectively, t is time, cr is the product of capacity and resistance, and C and k are constants) it is evident that the data of equation (2) with the single parameter, k, are not adequate to enable the expression of equation (1) with the two parameters, k and C, except in the special case, $C = 0$, and even in this case it is obvious that t in equation (1) is not obtainable for every value of V by taking a fraction of cr for the same value of V from (2).

The theoretical value of Lapicque's ratio and therefore its meaning can be derived easily from equations (1) and (2) as follows (Blair, 1932). Putting $V = 2R$ these equations become, respectively,

$$\log 2 = kt + C \qquad (3),$$

and

$$2 = (crk)^{\frac{1}{crk-1}} \qquad (4),$$

which is satisfied on putting $crk = 2$. Therefore on substituting this value of k[1] in (3) it becomes,

$$\log_e 2 - C = \frac{2}{cr} t$$

which in the special case, $C = 0$, reduces to

$$t = 0.3466\, cr$$

which is close to Lapicque's value. The factor, 0.37, is, however, the average of values which are rather widely divergent (Lapicque, 1926), the reason for these divergences being that $C = 0$ very rarely experimentally. It is, usually, not greatly different from zero with nerve, however, so the approximate agreement of the theoretical and experimental values in this special case is not surprising (Eichler, 1933).

It is not generally true, however, that $C = 0$ approximately, so the use of any conversion factor may be very misleading. Aside from this question, it will be evident that the factor is merely the relation of the corresponding points, $V = 2R$, of the two curves and that it cannot be used to relate the curves at any other points. Moreover, this conclusion is independent of the particular equations used here for illustrating its meaning.

The particular data to which reference in this regard appears desirable are contained in a paper by Rosenblueth and Rioch (1933). In this paper are given several excitation curves obtained with condenser stimuli. These curves after conversion by means of Lapicque's factor are demonstrated not to conform to Hill's (1910) direct current equation and to equation (1) which has the same form (Blair, 1932), and it is concluded in consequence that these equations are not valid. The proper procedure, however, would have been to apply the condenser data directly to equation (2). The data being from tissues which have not been investigated in

[1] This procedure of taking the condenser k as valid for the direct current data has been justified experimentally on the frog's sciatic nerve (Blair, 1935a) which is the same tissue as that employed by Lapicque.

regard to whether their excitation curves conform to the general class, and the method being unusual, it appears desirable to test these curves now in the proper way.

In table 1 are given the data as they were published (p. 523) except that the durations have been reconverted to the experimental *cr* values by means of multiplication by 1/0.37. The curve designations A, B, etc.

TABLE 1

Measured and calculated voltages for the voltage-capacity curves of the nerve supply to the nictitating membrane, curves A and B; of the nerve supply to pilomotors in the tail, curve D; and of the vagus, E

The factors k are derived from graphs like figure 1. The voltage in each case for $cr = 10$ is taken as the rheobase, both measured and calculated.

cr	A		B		D		E	
	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.
	volts	*volts*	*volts*	*volts*	*volts*	*volts*	*volts*	*volts*
0.0297								
0.0405			82.0	61.8			40.2	33.6
0.0486			69.0	58.6				
0.0595							27.0	26.4
0.0703			50.0	46.2				
0.0811					39.0	39.1		
0.1	13.4	13.4	36.0	37.2	36.0	35.5	18.3	20.3
0.149	10.2	10.5						
0.2	8.8	9.0	26.5	26.0	28.0	27.0	14.5	14.8
0.248	8.0	7.9						
0.297	7.4	7.3	22.5	21.8	24.5	23.9		
0.405	6.5	6.3			22.0	22.0	11.0	11.8
0.486	6.0	6.0			20.5	21.0		
0.595	5.6	5.6			19.5	20.3		
0.703	5.3	5.2	16.0	16.3				
0.811	5.0	5.0			17.5	19.1	9.6	10.0
1.000	4.7	4.7	15.0	14.9				
1.185	4.3	4.5					9.2	9.4
2.000			12.5	13.0			8.7	8.8
10.000	3.0		10.5		15.0		7.5	
	$k = 4.17$		$k = 6.15$		$k = 13.4$		$k = 10.3$	

are as before. Curve C is omitted. The columns of calculated voltages were obtained by means of equation (2).

The method of applying equation (2) (Blair, 1932; 1934) is illustrated in figure 1. The constant, k, is determined by plotting the linear relation,

$$cr \log \frac{V}{R} = \frac{1}{k}\left(\log \frac{V}{R} + \log cr\right) \frac{+ \log k}{k}$$

which is equivalent to equation (2) and is obtained from it by taking logarithms of both sides and rearranging. In figure 1 the data of curve A are given in this way. The resulting curve should be a straight line with a slope $1/k$, and it should intercept the axis of abscissae at the point, $-\log k$, if equation (2) is valid. It will be seen that these conditions are satisfied in the figure quite well. The agreement of the data with the

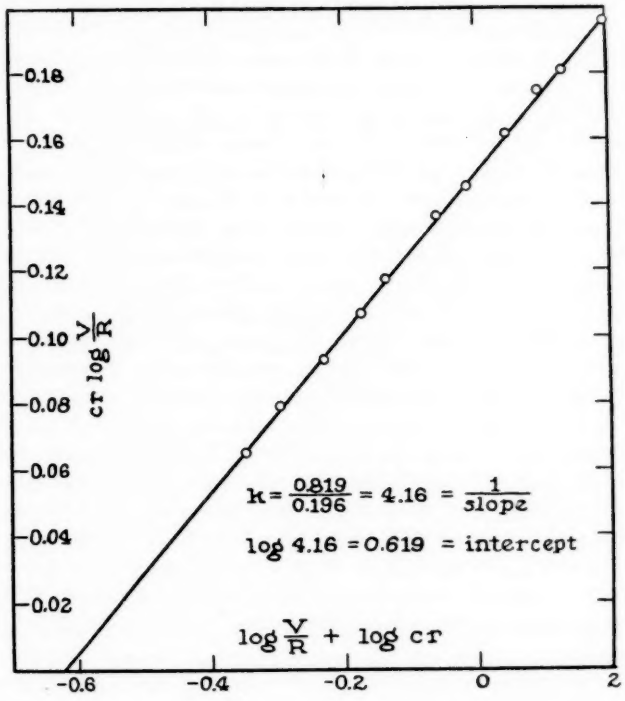

Fig. 1. The data of curve A plotted with $cr \log \frac{V}{R}$ as ordinates and $\log \frac{crV}{R}$ as abscissae in order to get k, which is given by the reciprocal of the slope and by minus the antilogarithm of the intercept on the axis of abscissae.

equation is more evident on comparison of the measured and the calculated voltages. These calculated voltages are obtained by substituting the value of k from the graph of figure 1 in equation (2) and solving for each value of cr. The measured rheobase is assumed to be correct for this purpose.

It will be seen that the calculated voltages of curve A agree quite well with the measured values. This is true also in curves B, D and E except

for the first two points of B and the first of E. Curve C did not conform sufficiently well to the equation to determine a line like figure 1 so it was omitted. It is quite possible, however, that this curve is a mixture of two or more curves.

All these data are from the anesthetized cat. Curves A and B are from the nerve supply of the nictitating membrane, C and D from the nerve supply to the pilomotors in the tail, and E is from the vagus. In each case repetitive stimuli were used, 2 per second, and the equivalence of the stimuli for different parts of the curve was judged by the obtaining of a given response, i.e., by a certain degree of contraction, of the bending of a particular hair, and of the slowing of the heart, respectively. As remarked by the authors it was not always possible to obtain the same response on repeating the same stimulus after an interval so that it is not unlikely that any given curve such as C may be poor because additional nerve fibers of different excitabilities are brought into action at some stage in the determination in order to get the required response.

No additional reason can be given for the disagreement of the upper points of curves B and E except that the voltage changes rapidly with the capacity in this region and a small error in the calibration of the condenser is important. It is not likely in any case that these divergences denote special properties of these tissues because the tissue of curve D, which fits well, is the same as that of C, which does not fit at all. Therefore, it seems permissible to conclude that these data of Rosenblueth and Rioch indicate, by their extensive agreement with equation (2), that the excitatory processes concerned in their experiments conform to the same laws as do others which have been investigated. On the basis of this conclusion it appears that their method of using a percentage of maximal response and repetitive stimuli on tissues which respond little or not at all to single shocks is sufficiently good to warrant its use in the study of comparative excitabilities.

It is noteworthy in regard to the excitabilities, using k conventionally as a measure, that they are of the order of 10. This is about the same as frog's muscle (e.g., Benoit's data—Blair, 1935b) while the frog's sciatic nerve gives values of the order of 1000 (Blair, 1935c) as does the ulnar nerve in man (Waller's data—Blair, 1932).

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THE EFFECT OF CO ON RECOVERY OF FROG SKELETAL MUSCLE

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An effect of CO on the respiration of skeletal muscle tissue has been described by Fenn and Cobb (1932a). They found that, when CO was substituted for the nitrogen in air, a 1½- to 3-fold increase in the rate of gas consumption occurred. (Schmitt and Scott (1934) have confirmed the presence of this increase.) The increase could not be correlated with any changes in the irritability or contractility of the muscle; it persisted when lactic acid formation was prevented by treatment with sodium bromacetate. The relation of the tension developed to the amount of initial heat produced was unaffected by the presence of 79 per cent CO. Since the anaerobic acid-base changes as followed by the absorption or elimination of carbon dioxide were parallel in the presence of CO and of nitrogen, CO was thought to be without significant influence on the breakdown of phosphocreatine or the formation of lactic acid. Fenn and Cobb (1932b) also established the interesting fact that the "excess respiration of CO" was actually due to a burning of the CO to CO_2 by the muscle, and that this burning could only be demonstrated in skeletal and heart muscle tissue.

The object of the work reported in the present paper was to investigate the effect of CO on the recovery process of the directly stimulated frog sartorius muscle. Comparative measurements were made in air and in a gas mixture of 79 per cent CO and 21 per cent O_2, *a*, of the excess gas consumption after stimulation; *b*, of the initial and delayed heat productions; *c*, of the initial and delayed heat productions after treatment with iodoacetic acid; *d*, of the rate of onset of fatigue; *e*, of the lactic acid content after a variable recovery period.

GAS CONSUMPTION MEASUREMENTS. *Method.* The gas consumption was measured in the Fenn respirometer, which was immersed in a water-bath set at 20°C. and remaining constant to approximately ±0.01°C. The respirometer used was specially modified to permit the change of gases in the experimental bottle and to allow the recording of the tension developed by the contracting muscle. The latter was arranged by means of a mercury "stuffing-box" of the type employed by Fischer (1930) and shown

by him to involve only negligible, if any, error in the measurement of oxygen consumption. The isometric lever used was of the torsion wire type and was supported by the stopper of the experimental bottle.

The procedure followed was to soak the dissected sartorius muscle in Ringer's fluid overnight in the ice-box. This was found to be necessary to ensure the "good condition" of the muscle, as evidenced by the low resting oxygen consumption of muscles so treated. The muscle was then mounted on the stopper, the electrodes were adjusted, and the tension lever was regulated to exactly zero tension. The volumeter, with the stopper in place was immersed in the water bath, and readings were taken for an hour or more until a uniform basal rate was established. The muscle was then stimulated, in the one set of experiments by 60 single shocks at the rate of 2 per second, and in the other set by a 1.5-second tetanus. Tension was recorded on a slowly moving kymograph drum. Rates of oxygen consumption were followed until a return to the resting level or to a new constant level was observed. The gas mixture was then changed, and the muscle was stimulated again. The third stimulation with the original gas mixture generally terminated the experiment. This technique was found to yield much more dependable results than simultaneous measurements on matched muscles placed in the different gas mixtures.

Results. Table 1 shows the results of experiments on three preparations chosen to include the range of variation encountered in the total of seven preparations used in investigating the twitch.

The excess gas consumption per gram tension in the CO-O_2 mixture is expressed in the last column in percentage of the normal as measured in air. While the magnitude of the decrease due to CO is seen to vary somewhat, each experiment of the seven performed showed uniformly less excess gas consumption for the same amount of tension developed when the muscle recovered in CO. The level of the resting gas consumption is, on the average, slightly higher after recovery for both CO and air. When air is readmitted, the CO effect is not completely reversible in all cases and was found to persist in about half of the experiments performed.

A second series of experiments, different only in that a 1.5-second tetanic stimulation was used, supplies the data in the lower section of table 1. In the three representative experiments shown, out of a total of seven experiments performed, the results with tetanic stimulation are seen to be substantially the same as those with the twitch; the muscle recovering in CO is found, in this case, also, to consume less excess gas than the same muscle recovering in air.

A summary of the twitch and tetanus gas consumption figures will be given in table 3.

MYOTHERMIC EXPERIMENTS. The purpose of these experiments was to establish the ratio of the total heat produced divided by the initial heat

TABLE 1

Excess gas consumption in air and in CO-O_2 gas mixture

EXP. NO.	GAS	T*	EXCESS GAS	EXCESS GAS PER GRAMS TENSION
Muscle stimulated with 2 shocks per sec. for 30 sec.				
		grams	*cu.mm.*	*per cent†*
1	Air	26.5	8.4	100
	CO-O_2	26.1	3.6	44
	Air	22.9	9.6	132
	Air	17.5	9.0	100
2	CO-O_2	18.7	5.4	56
	Air	16.2	6.6	79
3	Air	10.6	3.6	100
	CO-O_2	11.6	3.0	76
	Air	8.8	3.6	120
Muscle stimulated with a 1.5-sec. tetanus				
5	Air	33.0	27.0	100
	CO-O_2	36.0	12.0	41
	CO-O_2	33.0	17.0	63
	Air	19.5	16.8	105
7	Air	34.5	38.0	100
	CO-O_2	31.5	24.0	69
8	Air	27.6	32.0	100
	CO-O_2	23.3	18.0	67
	CO-O_2	15.9	11.0	60
	Air	6.3	5.2	71

TABLE 2

Heat measurements

GAS	T*	HEAT		TOTAL / INIT.	TL. / INIT.	DEL. HEAT
		Init.	Del.			
Muscle twitch—single preparation						
	grams	*gm.c m*				*per cent‡*
CO-O_2	33	16.2	13.2	1.81	7.8	62
CO-O_2	30	14.6	6.6	1.45	7.9	34
CO-O_2	29	13.0	6.2	1.48	8.6	33
CO-O_2	33	15.3	6.3	1.41	8.3	30
CO-O_2	28	14.4	7.0	1.49	7.6	39
CO-O_2	28	14.1	5.3	1.39	7.7	29
Air	29	14.6	19.0	2.30	7.6	102
Air	28	14.2	17.7	2.25	7.7	98
Muscle tetanus (0.5 sec.)						
Air	34.1	533	607	2.16	0.19	93
Air	34.1	520	700	2.26	0.20	107
CO-O_2	35.6	585	425	1.75	0.18	62
CO-O_2	31.1	454	256	1.56	0.21	43
Air	35.6	600	700	2.17	0.18	103
Muscle twitch after I.A.A.						
Air	59.6	36.8	34.1	1.93	6.50	97
Air	60	36.2	37.7	2.04	6.63	102
Air	59	34.8	36.0	2.04	6.72	102
CO-O_2	no	38.5	24.0	1.62		62
CO-O_2	tension	37.5	24.6	1.66		66
CO-O_2	record	42.8	32.7	1.76		76
CO-O_2		38.4	22.1	1.58		58
CO-O_2		30.4	15.1	1.50		50
CO-O_2		28.0	12.3	1.44		44

* Maximal tension in each twitch or average tension in each tetanus.

† In percentage of the excess gas consumption (per gram tension) in the first air period.

‡ Delayed heat in percentage of the average delayed heat in air per grams tension.

T = tension; Int. = initial; Del. = delayed;

$\frac{\text{Total}}{\text{Init.}}$ = total/initial heat; $\frac{\text{Tl}}{\text{Init.}}$ = tension x length/initial heat.

produced in each of the two gas mixtures used. If the diminished excess gas consumption for the CO muscle involves a decrease in the recovery oxygen consumed, it was to be expected that this ratio would likewise be diminished in CO, since the initial heat produced has been found to be unaffected by CO (Fenn and Cobb, 1932a).

Method. Hill's well-known myothermic technique was employed, a single frog sartorius preparation being used. A type Zd Kipp galvanometer was the current-measuring instrument, and the deflections were either photographed at a distance of 2 meters (for the muscle twitch) or read from a transparent Cambridge scale at the same distance (for the 0.5-second tetanus). Calibration by the condenser-discharge method was found to give the same results in air and in the CO-O_2 mixture.

As in the respirometer experiments, the gas in the thermopile chamber was changed so that determinations in the two gas media were made on the same muscle. Any experiment in which the heat base-line was irregular so as to cause ambiguity in its interpretation, was discarded.

Results. Table 2 shows the results obtained in a representative experiment chosen from a total of nine experiments performed with the twitch (a summary of these experiments is found in tables 3 and 4). The next to last column in the table shows the ratio of tension in grams times length in centimeters divided by the initial heat in gram-centimeters. Confirming the findings of Fenn and Cobb, referred to above, it is seen that CO has no effect on the development of tension or on the energy-producing reactions associated with it.

The ratio of total heat produced divided by initial heat produced is noted in the fifth column of the table. The average values of the ratio for this experiment are 2.27 for the muscle in air and 1.50 for the CO muscle. CO has therefore caused a 60 per cent decrease in the delayed heat production.

The middle section of table 2 shows the heat produced in a 0.5-second tetanus. The last column in the table shows the recovery heat production in percentage of the value determined for air. Conforming to the measurements on the twitch, about half of the recovery heat is missing in CO.

HEAT PRODUCTION AFTER IODOACETIC ACID. *Method.* The sartorius muscle was soaked in 1:25,000 parts of I.A.A. in Ringer's fluid for exactly 1 hour. The Ringer's fluid was withdrawn and after about 25 minutes for equilibration in the thermopile chamber, measurements were made of the heat production associated with a single twitch. The galvanometer deflection was photographed as before. The base-line was found to be quite constant in these experiments, and the deflections measured on the photographic record had a maximum height of 6 to 10 cm.

The concentration of iodoacetic acid used was described by Cattell and others (1931) as adequately preventing the formation of lactic acid but as

having no effect on the oxidations as such. In the experiments reported here every muscle showed a typical I.A.A. contracture when electrocuted before calibration.

Results. Confirming the work of Cattell and others, who found that the ratio of total heat produced, divided by the initial heat produced in the tetanus, is unchanged by soaking the muscle in I.A.A., these experiments show that this ratio for the twitch in air is equal to an average value of

TABLE 3

Average excess oxygen consumption and delayed heat in CO-O_2 mixtures in percentage of the corresponding value in air

	TETANUS		TWITCH	
	Observations	Mean	Observations	Mean
		per cent		*per cent*
O_2 consumption, av.:				
Best	10	63.9 ± 2.1	4	62.0 ± 4.5
Lowest	10	57.8 ± 3.6	4	54.8 ± 5.7
Highest	10	65.7 ± 2.5	4	65.2 ± 6.4
Delayed heat, av.	35	42.2 ± 2.0	18	52.0 ± 2.7

The figures show the probable error of the mean calculated according to the following formula:

$$R = \pm 0.6745 \sqrt{\frac{\Sigma (v^2)}{n (n-1)}}$$

TABLE 4

Summary of heat

	NO. OF PREPARATIONS	NUMBER OF DETERMINATIONS		AV. $\frac{\text{TOTAL H}}{\text{INITIAL H}}$	
		Air	CO	Air	CO
Tetanus	10	10	18	2.28	1.66
Twitch	9	16	35	2.06	1.45
I.A.A. twitch	6	7	36	1.98	1.42

1.98, which is well within the limits of the total heat/initial heat ratio for untreated muscles. Table 2 gives a single experiment in detail, and table 4 includes a summary of the experiments on six preparations.

Tables 2 and 4 show that the muscle which had been treated to prevent the formation of lactic acid still showed the diminished delayed heat production in CO to the same extent as the untreated muscle.

FATIGUE EXPERIMENTS. *Method.* A chamber was constructed to allow the change of gases and the admission of Ringer's fluid. Isometric tension

was recorded on a slowly moving kymograph drum. Both the make and the break shocks of an inductorium were used, the coils being adjusted so that both shocks gave an equal and maximal response. The muscle was stimulated directly, at the rate of 2 shocks per minute for periods of 1 to 2 hours. After the lapse of 2 minutes to allow the introduction of the other gas medium, stimulation was continued for another long period. The gas was exchanged at least three times in each experiment, air sometimes being used first and sometimes the CO-O_2 mixture.

Experiments with matched muscles, one in CO-O_2, the other in air, yielded inconclusive results on account of the inevitable variability in two apparently similar preparations.

Results. Nine preparations were used. The loss of tension in these experiments seemed to be quite independent of the presence or absence of CO. There was certainly no evidence of a more rapid loss of tension due to the accumulation of unoxidized end-products in the muscle while in CO. These fatigue experiments, therefore, seem to offer some support to the interpretation that, in spite of the diminished excess gas consumption and delayed heat production, recovery was more or less complete for the CO muscle.

LACTIC ACID EXPERIMENTS. Since it was thought that chemical analysis of the concentration of lactic acid would give a more reliable index of the degree of recovery after contraction than fatigue measurements, the lactic acid content of muscles which had recovered in air for a certain length of time was compared with that of muscles recovering in the CO-O_2 mixture.

Method. Matched muscles of the frog were used, the sartorius, the semitendinosus, and the tibialis anticus being employed on account of their thinness and the consequent assurance of an adequate oxygen supply. After dissection, they were soaked in aerated Ringer (in the icebox) for at least 6 hours. Each set of three muscles was then mounted in a separate stimulating chamber, one set in the CO-O_2 mixture, the other in air. After allowing 45 minutes to ensure the diffusion of gases into the muscles, they were each stimulated with a 30-second tetanus. Following exactly equal periods of recovery, the muscles were plunged in trichloracetic acid and ground up with quartz sand in the icebox. The reducing substances were removed with copper sulfate and calcium hydroxide, and the two sets of muscles were analyzed for lactic acid according to the technique described by Wendel (1933); 350 mgm. of tissue was the minimum amount used.

Results. The results of the lactic acid analyses, after variable periods of recovery, are listed in table 5. In considering these results, it should be borne in mind that several factors contribute to cause a large normal variability. The initial resting lactic acid content may vary, as shown by two experiments in which the muscles were prepared as usual, but were

not stimulated. The muscles in air had a resting lactic acid content of 25 and 12 mgm. per cent; in CO-O_2, 26 and 19 mgm. per cent respectively.

A second factor may lie in unequal stimulation of the muscles in spite of the care observed in placing them on the electrodes at equal lengths, and in keeping the time of stimulus as nearly equal as possible. A third factor which is probably considerable is the different rates of recovery of two matched muscles, of which ample evidence is found in respirometer experiments.

In view of this large normal variability, these data can only be interpreted as indicating that there was no marked accumulation of lactic acid in the muscles which recovered in CO.

Discussion. Since Warburg (1927) described an inhibitory effect of CO on the respiration of yeast, a number of other investigators have reported similar inhibitory effects on a wide range of organisms (this work is reviewed by Fenn and Cobb, 1932a). There are, in particular, two pieces of work which seem, superficially, to be very closely related to the type of investigation reported in this paper.

TABLE 5

Average lactic acid content after recovery from 30-sec. tetanus

NUMBER OF DETERMINATIONS	RECOVERY TIME	LACTIC ACID		HLa_{CO}-HLa_{air}
		Air	CO-O_2	
	minutes	*mgm. per cent*		*mgm. per cent*
2	2	92	82	−10
2	5	74	82	+8
1	15	76	85	+9
2	30	82	81	−1
2	60	75	73	−2
5	120	43	49	+6
2	180	56	59	+3
1	210	43	51	+8

Runnström (1930) finds no effect of CO on the respiration of the unfertilized sea-urchin egg, but a marked inhibition of the oxidation processes of the fertilized egg. Similarly, Bodine and Boell (1934) report that, while CO has no effect on the oxygen consumption of the grasshopper embryo in diapause, the inhibitory effect of CO on the respiration of the physiologically active cells is quite definite.

There seems to be a superficial analogy between these two instances and that of frog skeletal muscle; i.e., there is no apparent effect of CO on the respiration of the resting frog muscle (exclusive of the superimposed burning of CO), but a marked diminution of the oxidative processes following stimulation.

In general, the effect of CO has been described as inhibitory and has been explained as a poisoning or saturation of the enzyme surface. The effect on the recovery of frog skeletal muscle, on the contrary, does not seem to be of such a nature, since evidence for the accumulation of unoxidized products of activity, which might be expected if the action of CO were to inhibit oxidation, cannot be found either in the fatigue experiments or the lactic acid analyses. Furthermore, the fact that, when lactic acid formation is prevented by treatment with I.A.A., the effect of CO not only persists, but is unchanged in amount, shows that any specific effect on the oxidation of lactic acid is out of the question.

Unless evidence can be found showing the accumulation of the end-products of some as yet unidentified anaerobic, energy-producing, reaction, we must conclude, on the basis of the lactic acid and fatigue experiments, that recovery is almost or entirely complete in the CO muscle and that the essential nature of this effect of CO on the recovery processes of frog muscle is not specifically inhibitory.

These tentative conclusions may be tested in part by consideration of the data on excess gas consumption and on muscle heat. If we assume 100 per cent recovery of the muscle in air, we should expect that all of the energy-producing, anaerobic reactions would be aerobically reversed and that the energy as measured from the oxygen consumption would be therefore equal to the total heat measured in an isometric contraction. Accordingly, we may imagine a block, the total area of which may be considered equal to the total heat or the total oxidative energy per gram of tension developed in air, a certain portion representing the part of this total energy which appears as delayed heat, experimentally determined as 56 ± 3.0 per cent.

If we further assume that all of the excess gas consumed in the CO muscle is oxygen,[1] and that the energy evolved per mole of oxygen burned is the same in both muscles, we can imagine for the CO muscle a similar block representing the oxidative energy per gram tension developed, the area of which will be only 63.9 ± 2.1 per cent of the block for the air muscle, bearing the same relation as their excess gas consumptions. A third block may be constructed to show the total heat in CO, which may be calculated from the data in table 4 as $\frac{1.66 \pm 0.057}{2.28 \pm 0.059} \times 100$, or 73 ± 3.1 per cent of the total heat in air.

Since the initial heat per gram tension has been shown to be equal for the air and the CO muscle, we may represent the initial heat in the CO block as 44 ± 2.9 per cent of the total heat in air. This leaves a delayed heat of 29 ± 4.2 per cent (73–44), which has been experimentally measured as the wasted fraction of the oxidative energy. The remainder of the oxidative energy is used for resynthesis and is equal to 35 ± 4.7 per cent ($63.9 - 29$) of the total oxidative energy in air. Since, during breakdown, energy was evolved equal to 44 ± 2.9 per cent and for reversal during

[1] Although the assumption that all the excess gas consumption in the CO-O_2 muscle is oxygen seems quite probable, there is no experimental evidence that this is the case. If CO burning were increased by stimulation the excess energy available for recovery is still less than 76 per cent; if it were decreased the energy for recovery might be equal to the normal.

resynthesis there is available only 35 ± 4.7 per cent of the total oxidative energy in air, the recovery in CO may be estimated as 79.5 ± 11.8 per cent complete.

This calculation would indicate that recovery was incomplete and the size of the probable error would mean roughly that if five similar series of determinations of percentage recovery were made, it would be probable that four of them would show a recovery of less than 100 per cent in CO and that there would be a 1:1 chance that they would show a recovery between 67.7 and 91.3 per cent.

Assuming these figures to be correct, the efficiency of recovery may be calculated as $44/100 = 44$ per cent, for the normal muscle, and $35/64 = 55$ per cent, for the CO muscle. The results indicate therefore that CO increases the efficiency of recovery but may leave a small residue of incomplete recovery.

The preceding calculation would indicate a recovery of about 80 per cent. Turning back to a consideration of table 5, the trend towards slightly higher lactic acid concentrations after 2 hours or more of recovery might be considered significant of the small unpaid oxygen debt implied in the missing 20 per cent of recovery.

The principal effect of CO on the recovery processes of frog muscle would seem, therefore, to be that of increasing the efficiency of these oxidative processes, since the amount of oxygen consumed per milligram of lactic acid removed is less in CO than in air. While the mechanism of this effect is not understood, certain characteristic features should be mentioned. The effect may be completely or only partially reversible. In general, it may be said that the effect is more likely to be completely reversible when the muscle is in good condition. A second important feature is that the effect does not seem to be specific for the lactic acid oxidizing system, since the oxidative processes in the I.A.A. muscle are likewise affected.

In conclusion, it seems probable that an explanation of the exact mechanism of the action of CO on the recovery of frog skeletal muscle must await a more complete understanding of the relation between the chemical and energy-producing reactions concerned.

SUMMARY

1. When a stimulated frog muscle recovers in a mixture of 79 per cent CO-21 per cent O_2, it shows a diminished excess oxygen consumption and diminished recovery heat production when compared to a muscle in air.

2. A stimulated muscle fatigues at the same rate in either the CO-O_2 mixture or in air.

3. Chemical analysis after recovery from stimulation shows no markedly larger concentration of lactic acid in the muscle recovering in CO. It is therefore concluded that the principal effect of CO on recovery is not inhibition of lactic acid oxidation. Further evidence of this is submitted in that the effect of CO persists undiminished in the I.A.A.-treated muscle.

4. The principal effect of CO is described as an increase in the efficiency of recovery processes of frog skeletal muscle.

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BLOOD LIPIDS DURING PREGNANCY IN GUINEA PIGS

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There are few available reports concerning the effect of pregnancy upon the concentration of blood lipids in guinea pigs. In 1907 Oshima (1) noted that the blood of the mother contained many ultramicroscopic particles or chylomicrons while that of the fetus contained few. In 1910 Kreidl and Neumann (2) presented further evidence to the contrary conclusion and in the same year Kreidl and Donath (3) found that the weight of extractable lipids was actually greater in fetal than in maternal serum. It is possible that variations in diet were responsible for these apparent discrepancies; by analyzing blood from animals fasted overnight, our results have confirmed those of Oshima. None of these authors, however, determined the chylomicron count or extractable lipid at stages during pregnancy in the mother or in non-pregnant controls. Maynard, Harrison and McCay (4) state that Metzger, in unpublished experiments, found certain lipids to be increased in value during pregnancy in this species. There are no available data on the values of all blood lipids in both plasma and the red blood cells during gestation in guinea pigs.

Nevertheless this species presents features which suggested that such information might be of value in elucidating the causes of the well known change in lipid metabolism during pregnancy in man. Gestation lasts for considerably longer in guinea pigs than in most small laboratory animals, being about 62 days as compared with 32 days in the rabbit, for example. As a result, the young are at birth relatively mature, being capable of independent feeding and not dependent upon the mother's milk. The placenta is analogous macroscopically and microscopically to the human placenta. The gravid doe may be castrated during the latter half or two-thirds of pregnancy without abortion ensuing (5). There are no changes in the lipid composition of the ovaries suggestive of changes in physiological activity (5) such as occur in rabbits (6) in which gestation is accompanied by a decreased concentration of blood lipids or a ~~lipemia~~ lipopenia (7). Many analogies exist, thus, between gestation in man and that in guinea pigs and if one or more of these or other undiscovered common factors are responsible for the production of the lipemia of pregnancy in

man (8), then one might expect similarly to encounter a lipemia during pregnancy in guinea pigs. For these reasons an investigation has been made of the lipid values in plasma and in the red blood cells of gravid guinea pigs.

PROCEDURE. A colony of mature, virgin, female guinea pigs was isolated and each animal subjected to daily vaginal inspection. Oestrus in this species is readily detected by the opening of the vagina, which remains patent for 3 days and closes during the 2 weeks of the mid-oestral period. As each doe passed into heat it was put into a separate cage and mated with a healthy buck for 3 days. Two to 3 weeks later the female was again examined bimanually in an effort to palpate an early gravid uterus. If oestrus reappeared, the mating was considered unfruitful and the animal returned to the original colony.

Blood for lipid analysis was obtained by cardiac puncture with the animal under light ether narcosis. The animals were fasted overnight before drawing blood; Oshima (1) demonstrated many years ago that the fat particles of blood increase in number after a meal in the gravid guinea pig. Since the concentration of lipids in the plasma was found to be relatively low, an effort was made to obtain 15 to 25 cc. of blood. Frequently this resulted in the death of the animal. After centrifuging, samples of plasma and of the red blood cells were drawn off, extracted and analyzed for lipids by Bloor oxidative micromethods as modified by Boyd (10, 11, 12). The red blood cells, translucent from thorough packing, were removed from below the buffy layer of leucocytes and laked with a minimal amount of distilled water before adding the extracting fluid, alcohol-ether.

Blood was obtained at various periods during gestation. Early work revealed that there was little difference in the percentage of blood lipids at mid-pregnancy as compared with non-pregnant animals. Hence few samples were taken before the 30th day. Some animals examined at mid-pregnancy were re-examined at term. Others were examined at term for the first time to eliminate the possibility that bleeding itself might have produced the lipemia, as found by Bloor (9) in other species. Little difference was noted between these two types of term pregnancies.

RESULTS. The results obtained are shown in figures 1 to 5. The concentration of each lipid in the non-pregnant controls has been plotted in the left hand partition of each figure and spaced so as to give some conception of the variations encountered. The concentration of lipids in plasma and the red blood cells of normal guinea pigs has not been previously reported in extenso. The means and standard deviations (calculated as in a former paper (11)) found in this series of 10 non-pregnant female animals were:

		Plasma	*Red blood cells*
Total lipid.....	169	± 34 mgm. per cent	517 ± 60 mgm. per cent
Total fatty acid..	116	± 29 mgm. per cent	282 ± 36 mgm. per cent
Neutral fat.....	73	± 33 mgm. per cent	47 ± 28 mgm. per cent
Total cholesterol..	32	± 5 mgm. per cent	119 ± 19 mgm. per cent
Ester cholesterol..	21	± 4 mgm. per cent	8 ± 7 mgm. per cent
Free cholesterol...	11	± 2 mgm. per cent	112 ± 15 mgm. per cent
Phospholipid.....	51	± 12 mgm. per cent	347 ± 48 mgm. per cent
Ratio P/TC.....	1.66	± 0.47	
Ratio P/TC.....	5.0	± 1.7	
Ratio EC/TC.....	0.66	± 0.06	

Compared with human blood (8), guinea pig plasma contains about one-quarter as much lipid but the concentration of lipids in the red blood cells is about the same in both species. In guinea pigs, plasma neutral fat is one-half as great as in man, phospholipid one-quarter and the cholesterol fractions one-fifth to one-sixth. The ratios phospholipids to total cholesterol (P/TC) and to free cholesterol (P/FC) are higher than in man but the ratio ester cholesterol to total cholesterol (EC/TC) is about the same. The latter ratio was the only one which was more constant than its component lipids. Although these results were with female animals only, it is probable that they may be considered as normal values for guinea pigs in general; thus the values for cholesterol compare favorably with recent values reported in both male and female guinea pigs (13, 14).

Gestation produced a marked increase in the concentration of all plasma lipids except ester cholesterol. The lipemia did not occur until the latter half of pregnancy; as shown in figure 1, an abrupt rise occurred, beginning about the 30th to 35th days. The animals which survived bleeding at mid-pregnancy and were examined again at term showed increases in total lipid between three- and fivefold. On the average, total lipid may be concluded to increase about fourfold between mid-pregnancy and term. This increase is greater than that reported during pregnancy in any other species in which a lipemia occurs at this time. It is considerably greater than in man (8) which tends to disprove the theory of Baumann and Holly (15) that the lipemia of pregnancy in man is of toxic origin. Baumann and Holly (15) arrived at this theory by finding that pregnancy in animals (rabbits and dogs) is not accompanied by a lipemia; in view of the present results, it can no longer be said that man is the only species in which a lipemia occurs during pregnancy. In further support of their hypothesis, Baumann and Holly (15) stated, "It is well known that in the toxemias of pregnancy in man one always finds the cholesterol and phospholipid content of blood are very much higher than the values found in normal human pregnancies." Boyd (16) has shown that even in eclampsia the blood lipids need not necessarily be higher than in normal gravidae, but that the significant change is an increase in the P/TC ratio of plasma.

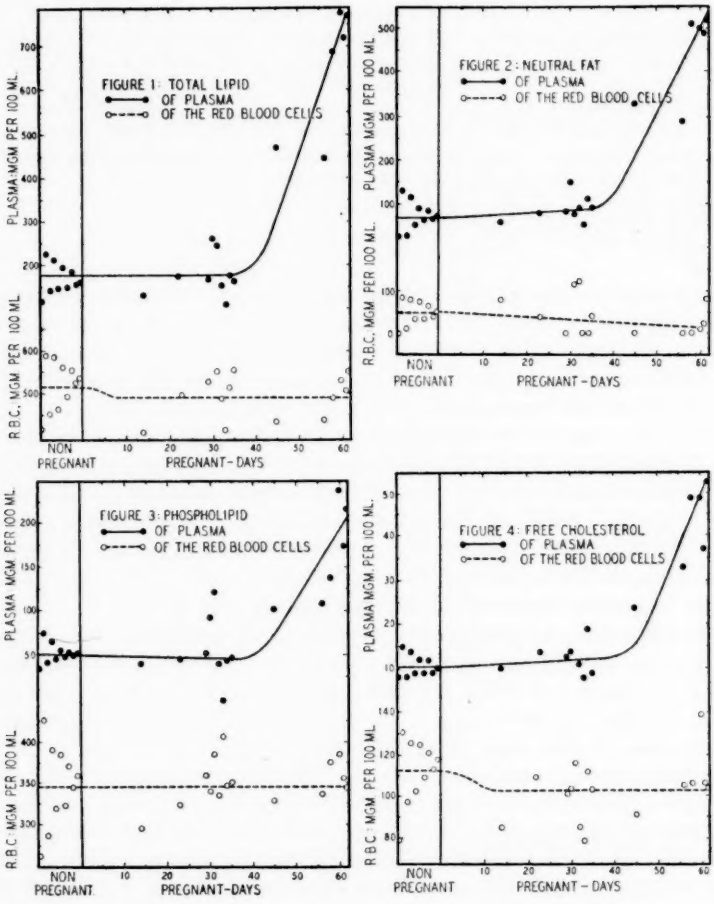

Fig. 1. The total lipid content of plasma and the red blood cells in normal and pregnant guinea pigs.

Fig. 2. The neutral fat content of plasma and the red blood cells in normal and pregnant guinea pigs.

Fig. 3. The phospholipid content of plasma and the red blood cells in normal and pregnant guinea pigs.

Fig. 4. The free cholesterol content of plasma and the red blood cells in normal and pregnant guinea pigs.

Values for neutral fat have been plotted in figure 2. Neutral fat increased relatively the greatest of all plasma lipids. In three cases analyzed both at mid-pregnancy and at term, it was 450 to 650 per cent higher

at the latter time. At mid-pregnancy there was no significant difference from normal but the concentration tended to be somewhat higher which is of interest in view of Bloor's (9) conclusions that neutral fat is the first plasma lipid to increase when a lipemia occurs. At term, plasma neutral fat averaged about 500 mgm. per cent as compared with about 100 mgm. per cent at mid-pregnancy and about 75 mgm. per cent in the non-pregnant controls.

As shown in figure 3, plasma phospholipid increased from about 50 mgm. per cent in the controls and at mid-pregnancy to about 200 mgm. per cent at term. Again the lipemic change was restricted to the latter half of pregnancy. The increase was not as marked as with neutral fat

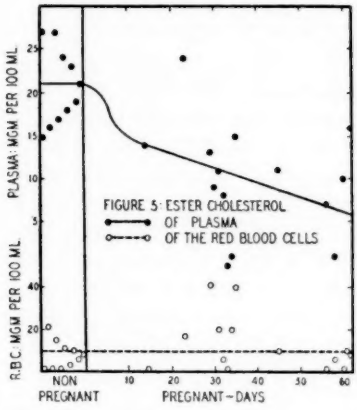

Fig. 5. The ester cholesterol content of plasma and the red blood cells in normal and pregnant guinea pigs.

and would appear to have occurred probably slightly after that of neutral fat. This latter is also the sequence described by Bloor (9) in the development of a lipemia.

The two fractions of plasma cholesterol were oppositely affected by pregnancy. The concentration of total cholesterol did not change greatly and has not been illustrated but free cholesterol (fig. 4) increased while ester cholesterol (fig. 5) decreased. The relative changes in plasma free cholesterol were similar to those of phospholipid which further bespeaks the well known close relationship of these two substances in vital economy. Thus free cholesterol rose from about 10 mgm. per cent in the controls and at mid-pregnancy to about 50 mgm. per cent at term, producing a curve very like that of phospholipid.

The curve for ester cholesterol (fig. 5) is interesting in that it was the

only one which declined during pregnancy. From the studies of Bloor (9), Maynard and his associates (4), Boyd (8) and others, it has appeared to be a rule that *all* plasma lipids vary in the *same* direction whenever variation occurs. This is indeed the common finding. Yet during the lipemia of pregnancy in guinea pigs, there was a progressive decrease in the concentration of plasma ester cholesterol throughout pregnancy. The decline was continuous from the beginning of gestation with no abrupt change at mid-pregnancy as occurred with the other lipids. The results bring to mind the unusual, unconfirmed findings of Gardner and Gainsborough (17) who recorded a decrease in plasma ester cholesterol in the second trimester of human pregnancy.

The concentration of each lipid in the red blood cells has been included as the lower interrupted curves in figures 1 to 5. The red blood cells were analyzed because Boyd (7, 8) has shown that changes in their lipid content are often entirely different to those in plasma and render determinations on whole blood alone difficult to interpret. The lipid content of the red blood cells has not been described with each lipid above because, as may be seen, there was no significant change in any lipid of these cells at any time during pregnancy. In a few instances a trend curve slightly below the mean of the controls appeared to fit best the several points but the changes could hardly be called statistically significant. Pregnancy in the human subject (8) is likewise accompanied by no significant variation in the lipid content of the erythrocyte. Aujaleu, Bugnard and Colombies (18) have reported low values for cholesterol in the red blood cells in human pregnancy but their differences were of an order similar to those herein found.

SUMMARY

The lipid composition of plasma and of the red blood cells was determined by oxidative micromethods in 15 pregnant and 10 non-pregnant guinea pigs. Up to the middle of gestation no significant change occurred but in the latter half a marked lipemia developed due to an increase in plasma lipids but no change in those of the red blood cells. In plasma there was approximately a fourfold increase in total lipid due to a fivefold increase in neutral fat, a fourfold increase in phospholipid and free cholesterol and a 50 per cent decrease in ester cholesterol. The decrease in ester cholesterol was continuous and progressive from early pregnancy.

These results indicate that changes in lipid metabolism during pregnancy in guinea pigs are analogous to those in human pregnancy. The results show that the human is not the only species in which gestation is accompanied by a lipemia and refute the validity of this argument when used to substantiate the claims of those who postulate that the lipemia of pregnancy in man is due to toxic causes.

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MEASUREMENT OF THE SUPERFICIAL TEMPERATURE GRADIENT IN MAN

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The experiments herein reported arose out of a study of the physiology of sensation. Detailed studies of the end-organs of the skin are few. There are more types than there are discretely recognized sensations (1). Clearly, more than one type of receptor may mediate the same sensation. This possibility has been suggested in the case of temperature sense. Head first emphasized the dual subjective character of warmth and of cold, and attempted to relate it to a double system of peripheral innervation. Some of our own observations appear explicable only on such a basis or on a system of peripheral or central interference of different sensations.

Preliminary observations were made on touch, pain, and temperature sensations in an attempt to test Head's theories of "protopathic" and "epicritic" sensibility, by correlating reaction times with physical and physiological variations (see reference to these experiments, Bazett, 1935) (2). The data obtained were inconclusive but demonstrated the necessity for more accurate standardizations of stimuli, in order to attain "equivalent" stimulation at different positions along the surface of a limb. Consequently a study of the normal resting subcutaneous temperature gradients was undertaken.

Bazett and McGlone (3) have published a small amount of consistent data showing the character of the gradient in the forearm. Since their determinations were incidental to a more extensive study of the temperature changes accompanying thermal stimulation in the arm, on a single experimental day they were only able to secure two values which were later used in the construction of a composite gradient curve.

In the present experiments their technique was altered in the interests of speed and economy, and the field of investigation was broadened to include other parts of the body. The thermocouples were threaded through the tissue in a manner which permitted the determination of gradients at a series of depths in a single experiment. Less sensitive galvanometers were used and special thermocouples were devised to obtain greater sensitivity with the recording instruments. Galvanometer sensitivity was sacrificed to permit more extensive accumulation of data to which statistical methods might be applied.

TECHNIQUE. Three subjects were used. The data detailed, however, are those of a single subject on whom the greatest number of observations were made. Two loop thermocouples were inserted alongside each other beneath the skin of either the deltoid region of the upper arm, the back of the wrist, the extensor aspect of the forearm or the back of the hand. The couples were a modification of the McGlone loop type (4), consisting of two fine wires, of constantin (gauge 41) and manganin (gauge 40), crossed and soldered at a single point. On one side of the solder junction the manganin wire was trimmed off very closely so that the constantin wire extended to serve as a tractor. On the other side, the two wires extended to heavier constantin and manganin wires (gauge 26) to which they were soldered, at a distance about 4 cm. from the thermocouple junction. In each case, the junction between light and heavy wires connected metals of similar thermoelectric properties. These two junctions were insulated in silk cloth and insulating varnish, and were then enclosed in a common cylindrical shield of silver foil to equalize chance temperature changes in the two. The heavy wires were continued for 30 to 60 cm. and terminated in cold junctions from which copper leads extended to the movable coil of a needle and scale galvanometer. The cold junctions were kept in paraffin oil in a thermos flask, and were held close to the bulb of a thermometer. The exposed junction was coated with insulating varnish. This type of thermocouple has a relatively small mass and therefore a small heat capacity at the exposed junction; furthermore it has a relatively low resistance which makes it quite sensitive even with a needle galvanometer. Critical tests have shown its accuracy to be as great as that of the McGlone loop type. The diagram of figure 1 illustrates the thermocouple and the arrangement of the apparatus. For further reference to the method see Bazett and McGlone (3).

The thermocouples were drawn into position under the skin by transfixing the tissue with a beading needle and drawing the tail of the thermocouple through in the eye of the needle. Two such needles were inserted, in a direction parallel with the long axis of the limb, at between 1 and 5 mm. distance from each other. Each needle emerged between 10 and 35 mm. beyond its point of insertion. The greatest depth at which either needle lay was from 1.5 to 3.5 mm. One was inserted from a distal to a proximal position and the other in the reverse direction. The two sets of skin punctures formed the corners of a rough parallelogram. The thermocouples were drawn into position and their paths were determined by x-ray; thus the depth of the junction could be determined at any point along the course beneath the skin, as the thermocouple was drawn from the entrance to the exit hole or vice versa.

Similar thermocouples were employed for measuring skin temperature. The tails of the surface couples were weighted down with artery clips to

assure close contact of their junctions with the skin. The measurement of skin temperature had to be confined to an area directly above the tissue position at which the sunken thermocouples were operating, since skin temperatures have been observed to vary as much as 0.5°C. at points less than 1 cm. apart.

Galvanometer deflections were observed over a period of hours while the extent of insertion of the sunken thermocouples was varied. Actual temperatures were then calculated from standardization values obtained

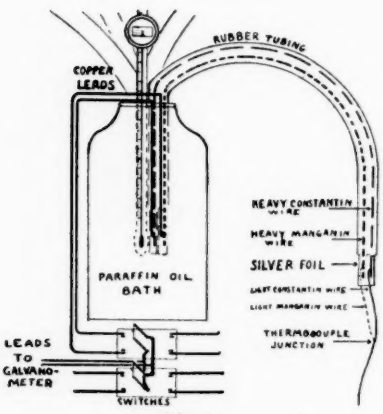

Fig. 1

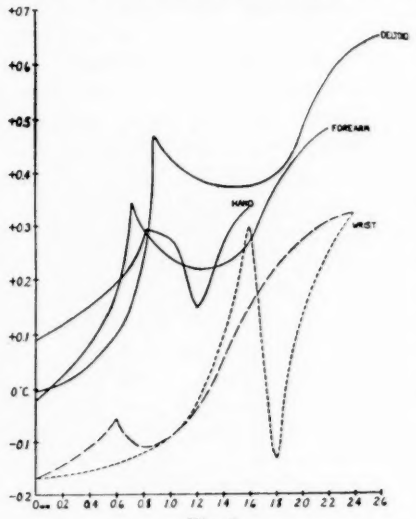

Fig. 2

Fig. 1. Diagram of thermocouple apparatus.

Fig. 2. Abscissae, depth in millimeter; ordinates, difference in temperature from mean of surface temperatures. Values on wrist inconstant; either curve equally possible; note temperatures at depths of less than 1 mm. in wrist all below surface temperature.

on the same day. Depths were calculated from measurement of the x-ray photographs. This gave series of temperatures and of depths from which the gradient could be determined, from the differences between mean skin temperatures and tissue temperatures for the various depths. Inaccuracies, due to unequal heat loss on the two sides of the thermocouple junction (since the tail was only a single wire while the leads consisted of two wires), were partially compensated since the value for any given depth that was used in the construction of curves was an average between values obtained in positions where the thermocouple was inserted to points both beyond and within its greatest depth.

RESULTS. Sufficient data could not be obtained for statistical accuracy, but the findings are significant. The types of gradient obtained are illustrated in figure 2 which shows the general differences. The results indicate that the type of gradient found by Bazett and McGlone in the forearm exists in the forearm, the back of the hand and the deltoid region of the upper arm. That is, in going from the surface of the skin into the tissue, the temperature rises to a critical value between 0.3 and 0.5°C. above skin temperature at depths between 0.7 and 0.9 mm., 0.8 and 1.1 mm., and 0.8 and 1.2 mm. respectively for the different regions. Going

TABLE 1

DATE	EFFECTIVE ROOM TEMPERATURE	AVERAGE SKIN TEMPERATURE		REGION	MEAN SKIN TEMPERATURE FOR REGION	
		Proximal position	Distal position		Proximal	Distal
	°C.	°C.	°C.		°C.	°C.
9/30/33	21.5	29.67	29.41	Back of hand	*29.24*	*29.16*
(9/ 1/33)	(22.2)	(29.80)	(30.78)			
8/17/33	23.3	29.48	29.54			
8/22/33	23.3	28.56	28.54			
8/30/33	22.5	33.11	33.44	Forearm near wrist	*32.02*	*32.38*
8/15/33	22.8	31.00	31.88			
8/23/33	23.7	32.04	32.11			
8/19/33	24.2	31.94	32.08			
8/31/33	21.7	33.14	32.55	Forearm near elbow	*32.44*	*32.12*
8/18/33	23.9	31.74	31.69			
(9/ 2/33)	(22.7)	(32.86)	(33.74)	Upper arm near shoulder	*33.40*	*33.02*
8/16/33	23.0	33.48	33.26			
8/21/33	23.2	32.54	32.26			
8/24/33	23.3	34.18	33.55			

Considerable reactive hyperemia on bracketed dates. Bracketed values omitted in estimating means.

still deeper, the temperature falls and another critical depth appears, only 0.1 to 0.4°C. above skin temperature, at depths between 1.1 and 1.3 mm., 1.2 and 1.3 mm., and 1.2 and 1.5 mm., respectively. At still deeper positions the temperature again rises and attains a value of more than 0.5°C. above skin temperature at depths beyond 2 mm. Over the back of the wrist there is less indication of critical depths as measured by this method with the small amount of data at hand, for the situation appears to be complicated in this area to an unusual extent by variations due to the nearness of relatively large blood vessels.

The data utilized for the determinations of the values here reported consisted of from 168 measurements on the forearm to 296 on the wrist. The irregularity of the results (especially in the case of the wrist) illustrates how, even with this volume of data, the issue may be complicated by extraneous factors. For real accuracy and adequate statistical treatment much more numerous observations would be required. Since at present no opportunity exists to obtain such data, the approximate curves are presented.

Skin temperature levels show more clear-cut distinctions: Average skin temperatures are listed in the table, showing the agreement between mean values for points on the skin from 0.6 to 2.0 cm. apart. In these particular experiments the skin temperature thermocouples were kept in the same positions on any one experimental day. It is seen in the table that skin temperature tends to be lower towards the joint of the limb. In addition there is a general tendency for the temperature of the skin to be higher nearer the trunk, as has long been recognized.

Discussion. The emphasis of this paper is upon the method, which permits the measurement of spot temperatures, and upon the apparatus, which is simple, inexpensive, and rather easily assembled. It has obvious advantages over previously described set-ups, which we have noted especially in the constancy of the skin temperatures as measured by several thermocouples simultaneously.

Hardy's general criticisms (5) against thermocouple measurements of skin temperature do not apply with any force to the method described here. He compared excellent radiometric apparatus with inaccurate thermoelectric elements which had large heat capacities, and concluded that thermocouple estimates of skin temperature can not be accurate to more than $\pm 1.0°C$. This conclusion is unwarranted since thermoelectric elements like ours, having small mass with little heat capacity, and partially imbedded in the skin by weighting the tail, are capable of accuracy to hundredths of a degree; with our rather insensitive galvanometers this absolute accuracy was probably within $\pm 0.2°C$. for single readings, with relative accuracy of $\pm 0.1°C$. in a series.

These experiments demonstrate that the type of gradient described for the more superficial layers of the skin by Bazett and McGlone can be established in a single individual, that such gradients are present in all parts of the skin so far examined, but that the exact character of these gradients varies in different parts of the body.

It is a privilege to acknowledge my indebtedness to Professor Bazett, to Doctor McGlone, and to the late Prof. T. H. Weisenburg. They have given most generously of their advice.

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SUMMARY

A method is described in detail for measuring skin and superficial tissue temperatures. Determinations of the superficial temperature gradients at several positions on the arm and hand are reported and the more suggestive findings are described.

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NORMAL DEXTROSE TOLERANCE CURVES, IN THE ABSENCE OF INSULIN, IN HYPOPHYSECTOMIZED-DEPANCREATIZED DOGS

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It has been shown previously (1) (2) that normal dextrose tolerance curves can be obtained in completely depancreatized dogs, which are receiving a constant intravenous injection of insulin plus dextrose just sufficient to maintain the blood sugar at a constant level. These results removed the necessity for the prevailing hypotheses which accounted for the characteristically normal disposal of administered sugar by assuming a mobilization of extra insulin from the pancreas. Our data also showed that, while the presence of the pancreas is not essential to the metabolic reactions which determine the normal dextrose tolerance curve, the presence and reaction of the liver are essential. We, accordingly, proposed an explanation of these phenomena based on a homeostatic mechanism of the liver, whereby this organ decreases its supply of sugar to the blood in response to an influx of exogenous sugar.

From this point of view the administered sugar may be regarded as the stimulus which activates the hepatic regulating mechanism, while the insulin in the circulating blood is merely one of the factors which determines the blood sugar level or threshold at which the homeostatic reaction comes into play. In the absence of insulin (pancreatic diabetes) the threshold is so high that the liver continues to pour sugar into the blood in the face of a marked hyperglycemia. An excess of insulin, on the other hand, lowers the threshold so that the normal blood sugar level becomes a sufficient stimulus to initiate the inhibitory mechanism; the supply of sugar to the blood is decreased, and hypoglycemia results.

The above considerations suggested the possibility that if, without the use of insulin, one could prevent the liver threshold from rising unduly after pancreatectomy, one should be able to obtain normal dextrose tolerance curves with the insulin factor entirely excluded. A suitable method seemed to present itself during a study of the dextrose tolerance curve in the hypophysectomized dog. We accordingly performed dextrose tolerance curves in completely hypophysectomized and depancreatized dogs, which

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had not received insulin at any time following the removal of the pancreas (1–8 weeks previously).

METHODS. Adult dogs were hypophysectomized by a method slightly modified from that of Dandy (3). Following complete recovery from the operative procedure of hypophysectomy, the animals were depancreatized. Glucose was administered intravenously until the animals were able to retain food, when they were placed on diets of lean meat, raw pancreas, and cane sugar, similar to those used in our previous studies on the depancreatized dog. No insulin was given at any time.

At intervals varying from 1 to 8 weeks after removal of the pancreas, and 18 hours after the last feeding, 1.75 gram dextrose per kilogram of body weight was administered intravenously. The blood sugar was determined during a control period and at intervals of 30, 60, 90 and 120 minutes after

TABLE 1

Normal dextrose tolerance tests in hypophysectomized-depancreatized dogs

DOG	TIME WITHOUT INSULIN	BLOOD SUGAR (MGM. PER CENT)				
		Before dextrose administration	After dextrose administration			
			30 min.	60 min.	90 min.	120 min.
	weeks					
1	1	375	650	460	314	371
2	2	542	719	654	527	528
3	3	371	550	437	395	376
4	4	384	557	459	430	388
5	6	362	519	534	440	350
6	8	415	467	422	356	368

sugar administration, on venous blood samples, by the Somogyi modification of the Shaffer-Hartman method.

RESULTS. About half of the animals tested yielded normal dextrose tolerance curves both as regards the maximum increase in the blood sugar level and the return to the pre-test level within two hours. The positive results are detailed in table 1, and graphically compared in figure 1. The curves in figure 1 were obtained by plotting the blood sugar values for each test as percentages of the respective initial blood sugar level for each test.

It will be noted that the control blood sugar levels in our animals ranged from 362 to 542 mgm. per cent. This is in accord with our previous observations (4) on the persistence of hyperglycemia in the *well-nourished* hypophysectomized-depancreatized dog. The influence of the height of the control blood sugar values upon the dextrose tolerance curves is shown by results such as are illustrated in figure 2. Graphs A and C (fig. 2) represent normal dextrose tolerance curves obtained at different times in a

hypophysectomized-depancreatized dog under the conditions described above. Graph B (fig. 2) represents a tolerance curve on the same animal at an intervening time, prior to which the control blood sugar level had been reduced towards the normal limits by fasting (4). It may be seen that this middle curve is quite abnormal as compared to those obtained at the higher control levels. It should be noted, however, that the maximum and especially the final blood sugar values of all the curves coincide rather closely.

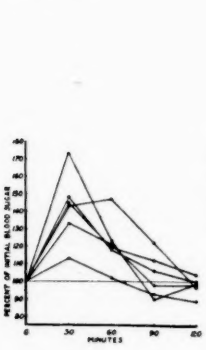

Fig. 1

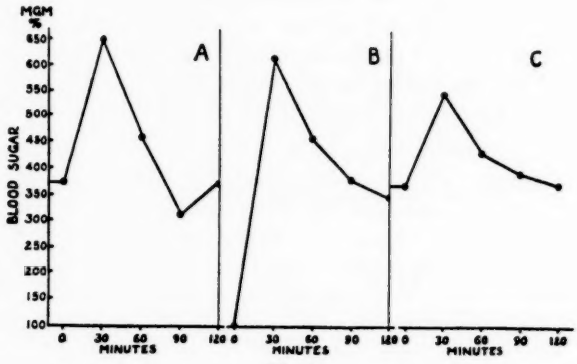

Fig. 2

Fig. 1. Normal dextrose tolerance curves obtained in completely hypophysectomized and depancreatized dogs, which had not received insulin at any time following the removal of the pancreas (1–8 weeks previously). The curves represent the tests detailed in table 1, but plotted as percentages of the respective initial blood sugar levels, for purposes of comparison.

Fig. 2. Dextrose tolerance curves obtained at different times in a hypophysectomized-depancreatized dog, showing the influence of the height of the initial blood sugar level upon the nature of the tolerance curve. The initial control blood sugars for curves A and C were at the level usually maintained by this animal in the post-absorptive state, when food intake was ample. Prior to the experiment in which curve B was obtained, the animal had been fasted until the blood sugar level fell to 100 mgm. per cent.

Discussion. Normal dextrose tolerance curves have been obtained in the absence of any known endogenous or exogenous supply of insulin.[2] This supports our previous results and conclusions as regards the erroneous nature of the hypotheses which account for the normal dextrose tolerance curve by assuming an extra secretion of insulin from the pancreas in response to sugar administration. The present results also remove the basis for these hypotheses which assume an activation or potentiation of insulin

[2] In a personal communication, Doctors Long and Lukens of the University of Pennsylvania have told us that they have obtained similar results in a "Houssay" cat.

which is already present in the circulation. We refer particularly to the recent hypothesis of Himsworth (5) to the effect that hyperglycemia is followed by the liberation from the liver of "insulin kinase" which then activates the previously inert insulin already present in the blood stream.

The fact that normal tolerance curves were obtained in our experiments supports the conception of the liver threshold from which these results were predicted, as outlined in our introductory remarks. This conception is further supported by the fact that normal tolerance curves were obtained at high initial blood sugar levels but not at low levels. It may be seen that, excluding the first rapid rise of the blood sugar in curve B (fig. 2) towards the initial height of the other curves, all three curves are strikingly similar. It is evident that the threshold for the homeostatic liver mechanism in this animal did not vary with the initial blood sugar level, but remained fixed. Once the blood sugar in curve B reached the threshold value, the normal response of the liver occurred. These considerations probably also explain the negative results obtained in some animals. It may be that in these animals the threshold, though lower than in the depancreatized animal with hypophysis intact, was still too high to be reached by the dose of sugar we used. The relation of the amount of sugar used for the test to the response obtained has been reported in connection with certain experiments on toxemia in a recent paper (6).

Our present results indicate that, in the endocrine balance which determines the threshold for the homeostatic mechanism, the hypophysis (possibly in conjunction with, or through the thyroid and adrenal glands) acts as an antagonist to the pancreas. When both these influences are removed, the threshold rests at the intermediate level which we have demonstrated. When adequate material for gluconeogenesis is available, the particular level of hyperglycemia which is maintained in the hypophysectomized-depancreatized animal depends upon and coincides with the liver threshold. Since this threshold is intermediate in height between the normal and the diabetic level, the hypophysectomized-depancreatized organism displays an ameliorated form of diabetes and a longer survival without insulin. The marked alleviation or disappearance of the diabetic manifestations which result from fasting these animals does not depend upon any change in the threshold of regulation but, as we have shown elsewhere (4), is due to a lack of noncarbohydrate sources for gluconeogenesis.

We have previously discussed (1) (7) the interpretation of various clinical disturbances in carbohydrate metabolism as disorders of the hepatic regulatory mechanism. Our present work indicates that the carbohydrate disturbances associated with the dysfunction of the hypophysis may be similarly explained.

CONCLUSIONS

1. Normal dextrose tolerance curves have been obtained in dogs, in which the presence of insulin has been entirely excluded.

2. These results oppose all pre-existing hypotheses which are based either on a reaction of the pancreas or an activation of insulin in response to sugar administration.

3. The occurrence of the normal dextrose tolerance curve is determined by a homeostatic reaction of the liver whereby this organ decreases its supply of sugar to the blood, in response to the influx of exogenous sugar. In this phenomenon, the rise in blood sugar may be regarded as the stimulus to the hepatic regulatory mechanism, while the pancreas and hypophysis are opposing influences which determine the blood sugar level or threshold at which the homeostatic reaction comes into play.

4. Clinical and experimental disturbances in carbohydrate metabolism due to hyper- or hypo-function of the pancreas or hypophysis, are best interpreted as disorders of the above liver mechanism.

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WORK CAPACITY IN THE RAT AFTER DESTRUCTION OF THE ADRENAL MEDULLA

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The intact gastrocnemius muscle of the normal rat (2) can sustain a high rate of work for several days, but this capacity is lost within a few hours after adrenalectomy (1). In partially adrenalectomized rats (4) large amounts of adrenal tissue are required for normal performance of work. These studies have not differentiated between the effects of removing the cortex and the medulla of the adrenal bodies. The rôle of the medulla in maintaining work capacity can be evaluated only by its complete destruction with a minimal damage to the cortex. This cannot be attained by direct ablation of the medulla. Jaffe (6), Wyman (8), and Pencharz, Olmsted and Giragossintz (7) have shown that in the autogenous transplant of the adrenals in the rat there is a differential survival of the two portions, the cortex remaining in good condition and the medulla undergoing degeneration. Ingle, in a preliminary study (3) verified the differential survival of the two parts of the adrenal when the whole gland was transplanted to the ovary. Experimental animals with the adrenal apparatus altered in this manner were employed here.

METHOD. Eighteen female rats were prepared with autogenous transplants of the adrenal glands. In a single stage, aseptic operation each adrenal gland was removed with its capsule intact, carefully cleared of fat and connective tissue and attached to the surface of the ovary by a single, fine silk ligature which passed through the body of each organ. As controls, a litter-mate of each transplant animal was subjected to a bilateral exposure of the adrenal bodies. A total of seven other litter-mates were completely adrenalectomized. No direct loss of body fluid occurred in any of the operations and post-operative complications were completely avoided. Each group of animals was allowed a delay period of forty days after the operation.

Upon completion of the delay period the transplant animal and its control were anesthetized with a standard dose of sodium luminal. The apparatus used for stimulation of the muscle, the recording of work, and other details of method have been described elsewhere (2). The gastrocnemius muscle loaded with 100 grams was made to contract three

times per second by direct faradic stimulation through silver needle electrodes. Each stimulus consisted of both the make and break shocks and was of optimum intensity. Six animals were worked simultaneously, the electrodes of each being connected in series with the others in the stimulating current thus allowing the same shock to stimulate each animal. The muscular contractions were registered on automatic work adders. The working animals were enclosed in a cabinet containing a water bath with temperature constant at 28°C. At eight-hour intervals the experimenter recorded rate of work, administered subcutaneously 0.5 cc. distilled water

TABLE 1

Work records of female rats having adrenals transplanted to the ovaries

WEIGHT		RATE PER MIN. AT 24 HOUR INTERVALS*						TOTAL REVOLUTIONS	
A†	B‡	0	24	48	72	96	120	T§	N‖
155	184	23	18	19	18	16	12	119845	117539
176	205	26	17	13	13	8	7	105120	42026
159	189	30	13	2				39746	83987
170	195	31	12	11	8	3		71182	56172
161	181	25	15	14	14	12	8	101900	30658
158	179	27	16	9	8	6	1	78736	62977
176	197	26	11	11	12	15	6	97265	75020
234	276	28	21	26	28	23	22	195530	156003
195	220	16	11	15	17	14	12	113588	163780
206	230	30	22	20	19	23	18	159940	156744
206	230	30	18	19	19	14	13	124950	118170
159	208	29	19	19	20	16	16	138970	76023
150	198	31	19	16	21	21	13	146676	102637
151	197	33	21	26	27	26	23	175710	100600
155	200	30	25	20	23	23	14	155868	132740
164	220	41	28	20	20	18	17	164172	164040
167	203	36	18	15	17	19	17	131470	106400
162	200	39	22	22	24	22	18	160700	105405

* Recorder revolutions each approximating 400 gram-centimeters work.
† Pre-operative.
‡ Post-operative, 40 days.
§ Transplant.
‖ Normal littermates matched for weight with transplants.

per 100 grams body weight and appropriate amounts of anesthetic. Stimulation was continued until the death of the animal or until 120 hours had elapsed. At necropsy the transplants were removed for histologic examination.[1]

RESULTS. The results in regard to weight changes subsequent to operation, the work performed and other observations are as follows (table 1).

[1] We are glad to acknowledge the assistance of Mr. George Butterfield, Department of Zoology, University of Minnesota, who carried out the histological examination of the glandular material.

1. Each of the seven completely adrenalectomized animals lost weight and succumbed within the forty-day delay period. All of the transplant group and their controls survived. Their weight gains were normal and no symptoms of adrenal insufficiency were apparent at any time.

2. In work performance the transplant animals showed a slight superiority to the normal controls. There is no reason to attach significance to the small difference noted since the transplant group was slightly favored in weight by the original selection.

3. Section of the transplanted glands showed that in each the medulla was apparently completely degenerated. Since a chromaffin stain was not employed here we have not demonstrated conclusively that all medullary tissue was absent along the inner border of the cortex. The cortex of the gland was normal in every case, the only degeneration being a small amount occurring at the point of ligature.

DISCUSSION. It is significant that adrenalectomized animals having their glands immediately replaced survive, gain weight, and withstand work, anesthesia, and the administration of distilled water in a completely normal manner. Animals treated in an identical manner except for the replacement of the gland develop overt symptoms of insufficiency and succumb in over 90 per cent of the cases studied in the laboratory (1) (3), and those animals which do survive have all been found markedly deficient in work capacity.

A delay period between operation and the work test is essential. Although the animal having autogenous transplants of the adrenal gland does not develop symptoms of insufficiency under optimum living conditions it remains deficient in its capacity to resist severe stress for periods up to thirty days (Ingle, unpublished studies). The transplanted gland establishes an elaborate blood supply between itself and the host tissue and it is probable that the cortex does not regain its normal functional capacity until this is completed. The site of the transplant is also highly important. In experimenting with methods it became clear that, although the adrenal gland would establish itself on the spleen, mesentery, in muscle, or subcutaneously sufficiently well to maintain life and normal body growth, these transplants could not be relied upon to maintain the animal for resistance to stress. Section of these transplanted glands revealed large amounts of necrotic cortical tissue and a meager blood supply. The vascularity of the ovary is a probable factor in making it a favorable host organ.

Ingle and Harris (5) have studied similar adrenal transplants with chromaffin stain and have found the medulla completely degenerated except for the infrequent occurrence of small groups of chromaffin cells on the inner border of the cortex. Extracts made from the transplanted glands were physiologically inactive when tested for epinephrine content. This finding seems to establish the methods employed here as adequate for

the experimental destruction of the medulla without the usual extensive destruction of the cortex.

The reasons for the differential survival of the two portions of the adrenal gland are unknown. The cortex is favored by direct contact with the host tissue and it may be unnecessary to assume that its survival and function at the time of degeneration of the medulla are due to any difference in the viability of the two tissues themselves.

The work capacity of the adrenalectomized rat can invariably be maintained at a normal or near-normal level by the administration of cortin. This observation provides strong evidence in itself that the muscular asthenia of adrenalectomized animals is due to the absence of the cortex only. However, the amounts demanded are large, and one is forced to consider the possibility that restoration with cortin alone involves some compensation for a medullary deficiency.

Insofar as our own interpretation of these results is concerned, we wish to limit it to the specific experimental situation which we have set up. It is conceivable that the violently exercising animal discharges sufficient amounts of epinephrine to increase work capacity. Our animals were not in a situation likely to elicit a reflex discharge of epinephrine. Also, it must be recognized that this experiment deals only with the non-essentiality of the adrenal medulla and not with other tissues which produce epinephrine or epinephrine-like substances.

SUMMARY

Eighteen female rats having autogenous transplants of the adrenal glands to the ovaries were compared with an equal number of sham-operated litter-mate controls for weight changes during a delay period of forty days and subsequent work capacity. Seven completely adrenalectomized animals were prepared but each developed overt symptoms of insufficiency and succumbed before completion of the delay. The transplant animals remained symptom-free, showed normal weight gains and normal work performance. Histological examination of the transplanted glands showed a surviving cortex but a degenerated medulla which was regarded as completely non-functional.

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VOLUNTARY ACTIVITY OF THE RAT AFTER DESTRUCTION OF THE ADRENAL MEDULLA

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The voluntary activity of rats in revolving drums is invariably reduced to a low level by complete bilateral adrenalectomy. Since loss of the adrenal cortex is followed by a state of profound muscular asthenia and by eventual death of the untreated animal, the accompanying loss of activity is not surprising or particularly relevant in determining the physiologic conditions of this trait of behavior. It has been demonstrated by Ingle, Hales, and Haslerud (4), that the capacity for muscular work is not diminished by loss of adrenal medullary tissue, but the effect of medullary deficiency on activity has not been studied. The autogenous transplant of the whole adrenal gland (2), (4), affords a convenient means of studying the problem since the cortical portion alone survives in the new locus.

METHOD. The levels of activity of thirty-four female rats, 90 to 110 days old, were determined by allowing them to run in revolving drums. The drums were 9.5 inches (24 cm.) in diameter, 8.5 inches (21.5 cm.) wide, and were equipped with Veeder counters which recorded one complete revolution either way. The experimental room was dark and nearly sound proof. The temperature varied from 22° to 27°C. The animals were fed a prepared, commercial mash containing 1.3 per cent sodium chloride, one part of which was mixed with from one to one and a half parts of water. A period of fifteen days was allowed for the animals to become adapted to the drums, after which records were taken for two consecutive periods of five days. The rank-difference correlation between these two periods was 0.855.

On the basis of these records the animals were divided as nearly as possible into two matched groups, each animal in the group to be operated on having a control with a comparable level of activity. Both adrenal glands of each animal in the experimental group were removed and transplanted to the surface of the corresponding ovary in a single-stage, aseptic operation. There were no postoperative complications. The control group of animals were not operated on. Animals were not placed in the revolving drums until eight days after the operation. Records of activity

were then taken for periods of five days until sixty-three days after operation.

At the conclusion of these studies on activity, the transplanted glands were removed from six of the experimental animals and the medullary portion was tested for the chromaffin reaction.[1] The rest of the animals in this experimental group were killed and the transplanted adrenal glands were extracted with physiologic saline solution. Similar extracts were made from normal adrenal glands and the two series of extracts were tested for the presence of epinephrine by determining their effect on the blood pressure of the dog, following intravenous injection, and on the perfused uterus of the virgin guinea pig.

RESULTS. All of the animals in the group whose adrenal glands had been transplanted survived operation and none showed symptoms of adrenal insufficiency throughout the period of experimentation. Gains in weight were normal.

Records of the activity of these animals following operation revealed that they were inactive during the first five-day period (ninth to fourteenth day after operation). The animals then returned to their pre-operative level of activity within twenty-eight days after operation. The control animals continued on a level slightly higher than this pre-operative level, but showed no significant variation from the level of activity for the experimental group of animals after the first twenty-eight days. Another expression of the trend of the data shows that nine of the seventeen animals whose adrenal glands had been transplanted exceeded their respective controls in activity during the pre-operative period; all experimental animals were lower in activity than their controls during the first five-day period after operation, while during the final five-day period nine animals in the experimental group again exceeded their respective controls in activity. The data on activity are summarized as averages in table 1.

Histologic examination of the six transplanted adrenal glands showed the cortices to be well preserved and normal. Degeneration had occurred only where the glands had been damaged by sutures. In all cases the medullary tissue appeared completely degenerated, but chromaffin stain revealed a small number of minute masses of medullary cells along the inner border of the cortex. They did not present the appearance of normal secretory cells and their total mass was too small to be of functional significance.

Saline extracts of the transplanted glands were negative for pressor effect when injected intravenously in the dog. Definite pressor effects were

[1] We are greatly indebted to Dr. R. W. Cragg, Section on Pathology, for the histologic examination of our material and to Dr. H. E. Essex, Section on Physiology, for the physiologic studies.

noted on injection of similarly prepared extracts from normal glands. Extracts from the transplanted glands had no inhibitory effect on the contractions of the perfused uterus of the virgin guinea pig. Definite inhibitory effects were noted following perfusion with small portions of extracts from normal adrenal glands.

Overt symptoms of adrenal insufficiency developed in each of the six animals whose transplanted adrenal glands had been aseptically removed after completion of the study of activity, and the animals died within twenty days.

COMMENT. It is clear from these experiments that the activity drive is not diminished by the experimental destruction of the adrenal medulla. The manifestation of this drive necessitates the expenditure of large amounts of muscular energy; thus our results support the findings of a more direct study of muscular work (4) after destruction of the adrenal medulla. Other studies have indicated that the adrenal medulla is not

TABLE 1

Records of activity expressed as the average number of complete revolutions of the drum

ANIMALS	NUMBER	BEFORE OPERATION, DAYS		AFTER OPERATION, DAYS		
		0 to 5	5 to 10	9 to 13	54 to 59	59 to 64
Experimental	17	30949	33393	13588	41798	42276
Control	17	30569	31765	35295	45455	40313

essential for activity: Bacq (1) performed bilateral sympathectomy in rats, a procedure intended to inactivate the medulla through denervation, and found voluntary activity undiminished after operation. Nice, Greenberg, and Greenberg (5) found that the administration of epinephrine chloride to adrenalectomized rats failed to raise the level of activity. One of us (Ingle) has confirmed this observation in an unpublished study. Administration of any form of epinephrine to the otherwise untreated adrenalectomized animal only hastens the onset of adrenal cortical insufficiency, although brief temporary benefits may be noted in some cases.

The delay in recovery of a normal level of activity after transplantation of the adrenal gland is similar to the latent period previously noted (4) for the recovery of work capacity. It is highly probable that there is hypofunction of the cortical tissue until a blood supply is established between it and the host tissue.

The dispensability of other possible epinephrine-producing tissues besides the adrenal glands has not been evaluated in this experiment.

SUMMARY

Normal levels of activity were established for thirty-four female rats by the revolving drum technic. On the basis of the preliminary records the animals were divided into two groups. Both adrenal glands for each of the experimental animals were excised and transplanted to the surface of the corresponding ovary. The control group of animals was not operated on. After a temporary depression in activity, the experimental animals whose glands had been transplanted returned to their pre-operative level, and twenty-eight days after operation equalled the activity of their respective controls. Histologic examination of the adrenal glands and physiologic tests for the presence of epinephrine demonstrated that the medulla of the transplanted glands had degenerated to a state in which it did not function whereas the cortical portion survived.

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CHANGES IN HEAT PRODUCTION AFTER REMOVAL OF MOTOR AND PREMOTOR AREAS IN MONKEYS

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Previous investigations (8, 9, 10, 13) indicate that lesions of the premotor area (area 6a, upper part, see Broadmann, 2) in primates give rise to a transient paresis of the extremities, while lesions of the motor areas (area 4) alone result in a primarily flaccid paralysis. Extirpation of both the motor and premotor areas causes an enduring spastic paralysis. In view of these findings it was of interest to determine whether there were any alterations in the basal heat production associated with the syndromes produced by these cortical lesions.

METHODS. The general plan of the research was to determine the basal heat production of the animals before and after each cortical lesion. Six immature rhesus monkeys (*Macaca mulatta*) and one adult capuchin (Cebus fatuellus) were used in this investigation. In four animals the motor and premotor areas were extirpated unilaterally at the first operation, and later in three cases, the motor and premotor areas of the other hemisphere. In order to disturb the feeding responses as little as possible the face area was left intact. Bilateral ablation of the premotor areas was performed in one animal and at a later date both motor areas were removed. In all other animals bilateral extirpation of the motor areas was undertaken.[2]

Metabolism was studied in an open-circuit system fashioned after the type devised at the Carnegie Nutrition Laboratory (3, 4). Full details of the technical procedure as carried out in this investigation are given elsewhere (17). The apparatus was tested intermittently during the research by burning alcohol in the chamber and by analyses of outdoor air. The mean respiratory quotients for alcohol in these check experiments was 0.664 ± 0.003, while the limits of variation from the theoretical of 0.667 were 0.674 and 0.658. The average results for the outdoor air analyses were 0.032 for CO_2 and 20.942 for O_2.

[1] This investigation was made while the author was a Porter Fellow of the American Physiological Society (1933–1934). The work was aided by a grant from the Research Fund of Yale University School of Medicine.

[2] The surgical procedures were performed by Dr. M. A. Kennard and Dr. J. F. Fulton.

The attempt was made to secure basal conditions for all subjects. All animals were at least 18 hours post-absorptive when under observation.

RESULTS. The results of the experiments are presented in four categories according to the type of cortical lesion.

TABLE 1

Changes in the respiratory metabolism of animals caused by unilateral ablation of areas 4 and 6

NO.	SEX	DATE	WEIGHT	O_2	R.Q.	CAL.	CHANGE	COMMENTS
			kgm.	*liters per hour*		*sq.m. per hour*	*per cent*	
2	♀	11/15	3.10	1.330	0.71	25.0		Control
		11/21						
		12/21						Extirpation left 4 and 6
		12/26	2.90	1.614	0.73	31.8	+27.2	Right side spastic
		1/29	2.80	1.442	0.71	29.2	+16.8	Right side spastic
		2/14	2.80	1.390	0.73	28.5	+14.0	Right side spastic
		2/28	2.80	1.223	0.76	25.0	0	No spasticity
1	♀	10/24						
		11/5	1.30	0.724	0.74	24.0		Control
		11/13						Extirpation left 4 and 6
		11/15	1.20	1.031	0.71	36.9	+54	Right side spastic
		12/18	1.25	0.802	0.71	27.4	+14	Slightly spastic
		12/21	1.25	0.719	0.77	25.0	0	No spasticity
3	♀	12/20						
		1/4	3.60	1.331	0.76	22.7		Control
		1/5						Extirpation left 4 and 6
		1/8	3.50	1.540	0.75	27.6	+21	Slightly spastic
		1/30	3.30	1.649	0.72	29.9	+31	No spasticity
		2/1	3.30	1.606	0.76	29.4	+29	No spasticity
		2/8	3.40	1.831	0.72	32.5	+43	
		3/14						Sacrificed miliary tuberculosis
4	♀	3/2						
		3/21	3.50	1.680	0.75	29.5		Control
		4/20						Extirpation left 4 and 6
		4/23	3.0	1.938	0.72	37.4	+37.4	Right side spastic
		5/8	2.95	1.979	0.80	39.3	+39.3	Right side spastic
		5/14						Animal sacrificed, miliary tuberculosis

A. *Unilateral extirpation of the motor and premotor areas.* Unilateral extirpation of the motor and premotor areas caused an increase in basal heat production of +21 to +54 per cent. These findings are presented in table 1. This increase occurred within the first week after operation and

metabolism remained above the basal level as long as spasticity was evident (nos. 1 and 2). Animals 3 and 4 developed tuberculosis during the period of observation.

B. *Bilateral extirpation of the motor areas.* The motor and part of the premotor areas were removed bilaterally in two animals. The results are presented in table 2. Both animals showed evidence of premotor syndrome, i.e., the extremities of no. 7 were for a time markedly spastic and forced

TABLE 2

Changes in the respiratory metabolism caused by bilateral ablation of areas 4 and the posterior part of area 6

NO.	SEX	DATE	WEIGHT	O_2	R.Q.	CAL.	CHANGE	COMMENT
			kgm.	*liters per hour*		*sq.m. per hour*	*per cent*	
5	♂	6/14	2.70	1.206	0.72	25.0		Control
		6/19						Bilateral extirpation area 4
		6/21	2.50	1.283	0.76	28.3	+13	Hind extremities spastic
		6/25	2.32	1.190	0.75	27.5	+10	Hind extremities spastic
7	♂	7/20	2.80	1.296	0.74	26.3		Control
		7/24						Bilateral extirpation area 4
		7/29	2.65	1.444	0.74	30.4	+16	Spastic
		7/31	2.65	1.448	0.75	30.5	+16	Spastic

TABLE 3

Changes in the respiratory metabolism caused by bilateral ablation of areas 6 followed by bilateral ablation of areas 4

NO.	SEX	DATE	WEIGHT	O_2	R.Q.	CAL.	CHANGE	COMMENT
			kgm.	*liters per hour*		*sq.m. per hour*	*per cent*	
6	♂	7/16	2.90	1.248	0.74	24.8		Control
		7/20						Bilateral extirpation area 6
		7/24	2.70	1.179	0.77	24.7	0	Spastic
		7/27						Bilateral extirpation area 4
		8/1	2.40	1.277	0.74	28.8	+17	Spastic
		8/3	2.40	1.238	0.79	28.2	+14	Spastic

grasping could be elicited from its left hand. Although the extremities of no. 5 were not as spastic as those of no. 7, it progressed with a distinctive gait. Heat production of no. 7 increased 16 per cent, from 26.3 to 30.5 cal.; this increase in no. 5 was less, 13 and 10 per cent on two determinations. The change in the latter case, however, is barely beyond the limits of error.

C. *Bilateral extirpation of the premotor areas.* Bilateral ablation of the

premotor areas was performed in one animal, no. 6; despite the transient spasticity which resulted there was no alteration in its metabolism. These findings are presented in table 3.

D. *Bilateral extirpation of the motor and premotor areas.* The results for four subjects are presented in tables 3 and 4. All bilateral lesions were made in two stages; in nos. 1, 2, and 3 unilateral extirpation of areas 4 and 6 of the left hemisphere was followed by a similar lesion in the right, while in no. 6 bilateral ablation of the premotor areas was followed by bilateral extirpation of the motor areas. The results were similar in all instances,

TABLE 4

Changes in the respiratory metabolism caused by bilateral ablation of areas 4 and 6

NO.	SEX	DATE	WEIGHT	O_2	R.Q.	CAL.	CHANGE	COMMENT
			kgm.	*liters per hour*		*sq.m. per hour*	*per cent*	
1	♀	12/21	1.25	0.719	0.77	25.0		
		12/30						Extirpation right 4 and 6
		1/5	1.10	0.838	0.80	32.3	+29	Markedly spastic
		1/11	1.10	0.758	0.76	28.9	+16	Markedly spastic
		2/15	1.05	0.887	0.72	34.1	+36	Regained some voluntary power on right side
		2/22	1.10	0.845	0.72	31.6	+27	Markedly spastic
2	♀	2/28	2.80	1.223	0.76	25.0		
		3/9						Extirpation right 4 and 6
		3/12	2.50	1.401	0.69	30.5	+22	Markedly spastic
		3/14	2.50	1.380	0.69	30.1	+20	Markedly spastic
		3/16	2.50	1.305	0.74	28.6	+14	Markedly spastic
3	♀	2/8	3.40	1.868	0.72	33.1		
		2/23						Extirpation right 4 and 6
		3/1	2.85	2.130	0.75	42.9	+29	Markedly spastic
		3/7	2.70	2.026	0.77	42.6	+29	Markedly spastic
		3/14						Sacrificed; miliary tuberculosis

i.e., an increase of 15 to 30 per cent in metabolic rate. Metabolism remained at this higher level as long as the animals were under observation (seven weeks in the case of no. 1). The extremities of all the animals showed the spasticity typical of these preparations. The results obtained on no. 3 were similar to the others but in view of its repiratory infection little significance is attached to the observations. Animals 5 and 7 gave similar results.

Discussion. The present experiments demonstrate an increased heat production and O_2 consumption associated with hypertonicity of the skele-

tal muscles. It is possible that physiological changes due to lesions of area 6a, other than spasticity, may have been associated with the observed increase in metabolic rate (14, 20). However no change in the heat production was observed in no. 6 following premotor extirpation. Furthermore there are three extraneous factors which may have influenced the findings: 1, change in body weight; 2, presence of respiratory or concurrent infection; 3, the effect of the surgical procedure *per se*. All animals lost some weight after operation despite special care in feeding. Benedict and others (1, 22) however have demonstrated that the basal metabolic rate falls during inanition, whereas an increase in metabolic rate was found in these experiments. Furthermore, results have been calculated in reference to surface area to minimize weight variations.

The presence of tuberculosis is precluded since post-mortem examinations were made at the termination of the experiments, and except in the

TABLE 5

The respiratory metabolism following section of the pituitary stalk

NO.	SEX	DATE	WEIGHT	O_2	R.Q.	CAL.	CHANGE	COMMENT
			kgm.	*liters per hour*		*sq.m. per hour*	*per cent*	
P.-1	♂	4/20	3.70	1.570	0.75	26.7		Control
		5/28						Pituitary stalk transected
		6/4	3.65	1.560	0.78	26.9	0	

instance already mentioned, no evidence of infection was noted. No. 6 developed a superficial skin infection of the scalp, but rectal temperatures were normal during observation and it is felt that the results were not significantly influenced.

The effect of surgical procedure has been studied in three monkeys which were observed before and after section of the pituitary stalk or a production of a small lesion in the hypothalamus (18). These procedures necessitated craniotomy and exposure of part of the cortex. Basal metabolic rate, determined at post-operative periods comparable with those reported here, was not altered (table 5).

Since the classical investigation of Parnas on the adductor muscle of the clam (15, 16) wide divergence of views has arisen as to the the influence of postural activity on the O_2 consumption (6, 12, 19). As the literature on this topic has been summarized in two excellent reviews (5, 7), consideration of previous investigation will not be given. The findings reported here confirm previous investigators who reported an increased O_2 consumption associated with an increase in postural activity (6, 11, 21).

SUMMARY AND CONCLUSIONS

1. The effect of extirpation of the motor and premotor cortex on the basal heat production of seven monkeys (6 rhesus monkeys and 1 capuchin) was studied. The metabolic rate of these animals increased significantly following the lesions.

2. Bilateral extirpation of the motor and premotor areas in three subjects was followed by an increase of +15 to +30 per cent. Two animals were studied following ablation of area 4 and the posterior part of area 6; increase in heat production of these animals was noted.

3. Bilateral extirpation of the premotor areas in one animal had no effect on the metabolic rate.

4. It is concluded that the increased heat production is associated with the spastic state resulting from the extirpation of areas 4 and 6.

It is a pleasure to acknowledge the aid and coöperation given by Dr. J. F. Fulton during this investigation.

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A QUANTITATIVE STUDY ON BLOOD CLOTTING: PROTHROMBIN FLUCTUATIONS UNDER EXPERIMENTAL CONDITIONS

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Prothrombin has been long recognized as an essential factor for the formation of thrombin, and yet from the physiological standpoint it has received little quantitative study. With methods outlined below we have established the normal level for prothrombin in plasma, and its fluctuations under certain experimental conditions. We have shown also that a surprisingly small portion of the prothrombin present is needed to form a normal clot.

Prothrombin is known only by its ability to form thrombin. For this reason its quantitative estimation depends upon biological assay methods. Howell (1) used the clotting time of recalcified plasma as a measure of the prothrombin present. Quick, Stanley-Brown and Bancroft (2) have improved the method by adding tissue extract to insure complete and prompt conversion of all prothrombin present, and they have presented interesting data with the aid of such technic. This total clotting time, however, is made up of the prothrombin conversion time and of the time required for the thrombin formed to react with the fibrinogen. The conversion time alone depends in an obscure way upon the prothrombin concentration and upon other variables of unpredictable importance. The thrombin phase overlaps the conversion phase to a variable degree and is itself a complex function of the amount of thrombin formed. The uncontrolled summation of these two reactions gives a clotting time which is very difficult to interpret in terms of prothrombin concentration. We feel that it is better to separate the two phases experimentally, and to use only the time required for the second phase as a measure of prothrombin. To do this one can transform the prothrombin to thrombin in a preliminary step; then the thrombin formed may be titrated by means of serial dilution technic. This technic, used by older workers and very recently by Eagle (3), permits one to determine the relative potency of various mixtures. In the past the pitfalls have been that often the prothrombin was partially lost or altered in preliminary purification, or was converted incompletely into thrombin. At times, too, the thrombin formed was allowed to dis-

integrate partially before being titrated. For these reasons the thrombin solutions were weak, and they represented a small and probably an extremely variable fraction of the total plasma prothrombin. Another difficulty is that the methods previously employed are purely relative and, without fixed standards, cannot be used to compare one day's results with those of another. We have taken steps to remedy these various defects. We have been careful to effect complete and rapid conversion of prothrombin to thrombin. Also, to prevent disintegration of thrombin formed, we make the serial dilutions prior to activating the prothrombin, instead of after the thrombin has been formed. The antithrombin is thus so dilute that ordinarily it does not interefere with the titration. Even with moderate dilution, antithrombin rapidly loses its effectiveness. The problem of fixed standards has been solved by using a standard clotting interval as a fixed point. The degree to which the unknown solution is diluted in reaching this point gives a measure of the amount of thrombin present. We are then able to express this amount in units of dilution.

In a recent article (4) we have discussed the preparation of reagents and laid in part the groundwork for the method now described in detail. An outline of the method and a number of our earlier results have been given in a preliminary report (5). The prothrombin of plasma can be converted completely into thrombin by adding an optimal amount of calcium and an excess of thromboplastin. For convenience in this work we have defined thrombin of unitary concentration as being of just sufficient strength so that in the presence of fibrinogen (0.08–0.10 per cent) it will form a clot in 15 seconds. The pH of the reagents is adjusted to 7.4 and the titration is carried out at a room temperature of 28°C. Prothrombin of unitary concentration is defined as being of sufficient strength so that when completely converted it will form an equal volume of unitary thrombin. Since we find that normal dog plasma must be diluted about 225 times to reduce its prothrombin concentration to unity, we speak of its concentration as being about 225 units. In experiments carried out over a period of several days we take the added precaution of doing parallel titrations on pooled plasma from several normal dogs. This eliminates slight errors due to variables such as room temperature and the reactivity of the fibrinogen.

The reagents used are prepared as follows: 1. Oxalated saline. Dissolve 0.075 gram $Na_2C_2O_4$ in 100 cc. saline (0.9 per cent NaCl). 2. Tissue extract (thromboplastin). Extract 0.3 gram of dry perfused dog lung with 10 cc. saline. Centrifugalize briefly. Use the supernatant fluid. 3. Oxalated plasma. Draw blood from the jugular vein of a dog into a vaselined syringe and place in a 15 cc. hematocrit tube containing 2 cc. of 1.5 per cent $Na_2C_2O_4$. Centrifugalize thirty minutes at 3000 r.p.m. Note the volume of plasma present in order to calculate the dilution by the anticoagulant. 4. Fibrinogen. Precipitate oxalated dog plasma by the

addition of one-third its volume of saturated $(NH_4)_2SO_4$. Dissolve the precipitate in a volume of oxalated saline equal to that of the plasma used. Repeat the process of precipitation twice. Dissolve the final precipitate in a volume of oxalated saline equal to one-third that of the orginal plasma. Dialyze against oxalated saline for ninety minutes in the ice box. Visking casing, manufactured by the Visking Corporation, Chicago, is used as the dialyzing membrane. 5. Calcium. Dissolve 0.5 gram $CaCl_2$ and 0.525 gram NaCl in 100 cc. H_2O. 6. Heparin. Use commercial heparin (Hynson, Westcott and Dunning).

Before the titration is carried out the plasma is defibrinated. At first this was done by heating to 56°C., but a variable destruction of prothrombin occurred. To avoid this we adopted the use of thrombin for the defibrination. This is prepared fresh by mixing 6 drops of saline and 3 drops each of calcium, tissue extract and plasma. The serum, rich in thrombin, is expressed and used at once. The following example will illustrate the defibrination and the subsequent titration of prothrombin in normal oxalated dog plasma. Hematocrit readings showed that each cubic centimeter of this plasma was diluted by the presence of 0.26 cc. of isotonic oxalate added to prevent clotting. Thirty drops of the plasma were now defibrinated by adding 3 drops of thrombin prepared as above. A clot formed in 18 seconds. After allowing 15 minutes to insure complete clotting and destruction of excess thrombin, the fibrin was rolled out. The expressed fluid, containing the prothrombin, was diluted 1–20, 1–30, and 1–40 with oxalated saline. When tested for thrombin these solutions did not clot fibrinogen. To test them for prothrombin we mixed 3 parts of each with 3 of saline, 3 of calcium, and 3 of tissue extract. After suitable periods of incubation we added 3 drops of fibrinogen to 12 drops of each incubated mixture. The clotting time was then measured with a stopwatch. From data already given, the total plasma dilution at the time of clotting can be calculated to be 1–149, 1–223, and 1–297 in the three dilutions used (see table 1). As shown in the table, we make it a practice to try a number of incubation periods, for in some plasmas the prothrombin is converted more slowly than in others. The thrombin concentration typically rises to a plateau which is maintained for a safe interval of time. In the above example, the plateau shows a 15 second clotting time with the plasma diluted 1–223. The prothrombin unit concentration in the undiluted plasma was, therefore, 223.

It is sometimes a tedious procedure to find the exact dilution which will produce precisely a 15 second clotting time at the beginning of the plateau. We, therefore, use an interpolation curve to correct for deviations of one or two seconds from the 15 second end-point.

Prothrombin utilization in clotting. Normal blood contains vastly more prothrombin than is actually needed to form a clot, for even when diluted

200 times sufficient thrombin may be produced to clot fibrogen in a few seconds, as in table 1. Here, of course, a large excess of thromboplastin was added, and the prothrombin was converted rapidly and completely into thrombin. In contrast, blood which clots spontaneously does so only by virtue of traces of tissue juice set free from injured tissues or from breakdown of blood cells and platelets. One sample, collected with reasonable care to minimize contamination, clotted during a 15 minute period of high speed centrifugalization. The serum, oxalated at once, was found to contain 220 units of prothrombin. A control titration on oxalated plasma from the same dog showed this to be 92 per cent of normal. As we have shown above, the 8 per cent consumed is itself many times the amount required to convert the fibrinogen into fibrin. Part of the unused thrombin is formed after clotting begins. Also, much thrombin, no doubt, is rendered ineffective by the antithrombin which is so abundant in undiluted plasma.

TABLE 1

The titration of prothrombin

FINAL PLASMA DILUTION	CLOTTING TIMES			
	30 second incubation	45 second incubation	60 second incubation	90 second incubation
	seconds	*seconds*	*seconds*	*seconds*
1–149	14	12	12.5	13
1–223	20	15	15	17
1–297	24	21	21	23

If clotted blood is allowed to stand for several hours the cells and platelets probably continue to give off thromboplastin. Also, in the presence of calcium, the tissue juice already present continues to act and the prothrombin content of the serum gradually falls. In one experiment considerable thromboplastin was present during clotting, and fresh serum obtained by brief centrifugalization contained only 54 per cent of its original prothrombin. Part of the serum which was left untreated with oxalate continued to form thrombin which, of course, was soon destroyed by the antithrombin present. At the end of three hours the serum contained the merest traces of thrombin and only about 5 per cent of the original prothrombin.

Oxalated plasma, when carefully collected, contains very little thromboplastic substance. It clots slowly when recalcified and, if tested at once, the serum is found to contain large amounts of prothrombin. One case, typical of many, clotted in 6 minutes on adding an optimal amount of calcium. Titration carried out at once showed 80 per cent of the prothrom-

bin to be present. A duplicate sample which was allowed to stand five hours before being oxalated was found to have only 20 per cent of its prothrombin left. In another experiment the initial contamination was deliberately increased by adding small amounts of lung extract. On adding calcium a clot formed in 34 seconds. The serum was reoxalated at once and found to contain less than 10 per cent of its original prothrombin. Prothrombin conversion in general is far in excess of minimum requirements. It continues long after the clot has formed. The speed at which it is converted depends in a striking way upon the amount of tissue juice available.

Normal values for plasma prothrombin. Table 2 shows the daily prothrombin fluctuations of normal dogs maintained on a mixed diet of table scraps. The average values for each dog show that the prothrombin levels are not identical. The highest dog (no. 3) showed unit values of 245, while the lowest dog (no. 4) showed only 200. The individual daily fluctuations

TABLE 2

Daily prothrombin titrations on normal dogs

DOG USED	PROTHROMBIN UNIT CONCENTRATION				
	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Average Sept. 5–8
Control pooled plasma (3 normal dogs)	204	227	218	218	217
No. 1	204	217	224	230	219
No. 2	212	206	221	227	216
No. 3	216	252	253	260	245
No. 4	186	208	198	209	200

were somewhat less than we sometimes see. Apparently, in this case, the reagents used in the titrations varied but little from day to day. Were this always true, a pooled plasma control would hardly be indicated. It is noteworthy that the daily fluctuations of pooled plasma were almost as great as those of the individual plasmas. This suggests that the small fluctuations are due mainly to technic rather than to variations in the animals themselves.

Further evidence of the stability of the prothrombin level was shown when we made gross changes in the diet. In one experiment the prothrombin values of four dogs were averaged and compared with those of pooled plasma from a constant group of dogs maintained on mixed diets. On table scraps the four dogs had average prothrombin values 98 per cent of the normal control. On a diet of lean beef (5 days) the average was 102. When maintained for 6 days on white bread the average was 96 per cent; 5 days of starvation gave values of 99. We feel that these variations lie

well within the limits of experimental error. Long continued abnormal diets might produce noteworthy changes, but the above experiment does show that minor changes in diet from day to day can be ignored in a general program of experimentation.

Prothrombin level with repeated hemorrhages and with plasmapheresis. A 20 kilogram dog, on a mixed diet, was bled 1880 cc. in five bleedings over a period of 12 days. The red cell hematocrit, 52 per cent by volume at the beginning, fell to a level of 27 per cent. It was kept at or below this level by the removal of a total of 1250 cc. of blood at intervals during the next month. The plasma prothrombin during the week prior to onset of bleeding varied between 89 and 101 per cent of normal pooled plasma, the average being 93 per cent. In the period of anemia there was no consistent change from day to day. During the last 13 days the prothrombin varied between 93 and 108 per cent, with an average of 95. Clearly, the prothrombin removed by hemorrhage is rapidly regenerated. Prothrombin readings taken on the day after hemorrhage showed no diminution below the general base line. Prothrombin thus resembles fibrinogen in being rapidly replaced following depletion. The ability of the animal to replace prothrombin rapidly was shown even more strikingly by plasmapheresis experiments carried out by the method of Holman, Mahoney and Whipple (6). In one, a 9 kilogram dog was maintained two months on a low protein diet, and then in the course of 6 weeks 6705 cc. of blood were removed and replaced by washed cells. Although the total plasma protein was below 3.5 per cent for the last 5 weeks, the prothrombin level showed no significant reduction—an average of 94 per cent compared with 101 per cent for the normal value on mixed diet.

Turpentine abscess. Distemper. Subcutaneous injection of 1 cc. of turpentine produced in a dog definite swelling and induration within 18 hours. The swelling increased, became fluctuant and drained to the surface on the seventh day. Healing was largely completed 5 days later. The plasma prothrombin, 108 per cent of pooled plasma prior to turpentine injection, fluctuated between extremes of 92 and 114 per cent, but showed no definite trend to correspond with the suppurative process. In contrast, the plasma fibrin determined by a modified Kjeldahl procedure (7) showed the rise so characteristic of suppuration (8). In this experiment the maximum of 725 mgm. per 100 cc. of plasma occurred just before rupture of the abscess. After rupture the value fell over a 9 day period to the original base-line of 275 mgm. per 100 cc.

Likewise, we have found no change in prothrombin level as a result of distemper, even though the infection was quite severe and the plasma fibrin was markedly elevated. The fact that these inflammatory conditions have no effect on plasma prothrombin, but do affect plasma fibrinogen, suggests that the production of these two clotting factors involves clearly dissociated processes.

Peptone plasma. Heparin plasma. India ink plasma. As Howell (9) has pointed out, peptone plasma is very much like normal plasma to which heparin has been added. In both there is a marked increase in antithrombin, and in both a great excess of thromboplastin is needed to convert the prothrombin to thrombin. Even with this excess of antithrombin, the plasmas are so much diluted in the titration of prothrombin that the antithrombin usually does not interfere with titration. However, with low prothrombin titres, and hence with plasmas less diluted, a little excess of antithrombin may destroy a significant amount of thrombin before the titration can be completed. Likewise, there may be difficulty with normal prothrombin titres in cases where a large excess of heparin is present. To obviate the difficulties in these extreme cases, we first treat the plasma with an equal volume of a saturated ammonium sulphate solution. The globulin precipitate contains very little antithrombin, but when redissolved and dialyzed against oxalated saline, it is found to contain 80 to 100 per cent of the original prothrombin.

The incoagulable plasma from three peptonized dogs showed 95, 100 and 98 per cent as much prothrombin as the normal pooled plasma, each titrated with the ammonium sulphate modification. Another peptone plasma, titrated without the use of ammonium sulphate, showed 96 per cent prothrombin. Clearly the antithrombin present did not interfere with the analysis in this case. It has been our experience that peptone plasma usually has an antithrombic activity comparable to normal plasma which has been treated with 1 mgm. of heparin to each 2 cc. of plasma, but rarely to heparin plasma of twice this strength. With strongly heparinized plasma we find it necessary to use the ammonium sulphate modification. We find normal prothrombin values, even though the plasma contains as much as 5 mgm. heparin per 1 cc.—an amount over ten times that needed to prevent clotting.

The intravenous injection of Higgins' American India ink (0.5 cc. per kgm.) yielded strongly antithrombic blood. Like peptone blood it was spontaneously incoagulable. The plasma prothrombin did fall moderately —about 20 per cent—in one typical experiment.

Chloroform and phosphorus poisoning. The chloroform dogs were first deprived of food for 24 hours. Then extensive liver necrosis was produced by keeping the dogs under moderately deep chloroform anesthesia for 90 minutes. The prothrombin remained normal for 8 to 15 hours, after which it fell rapidly to a very low level, usually under 10 per cent of normal. One dog, typical of the group, had normal prothrombin at the end of 10 hours, but at the fifteenth hour the level had fallen to 50 per cent of normal. By the end of 24 hours it was down to about 5 per cent, and remained at this level during the next 30 hours. The prothrombin then began to rise gradually, but was not back to normal until the end of the

seventh day. Other experiments have shown that by this time the injured liver would have been largely regenerated. The plasma fibrin rose several per cent during the first 10 hours, but between the twentieth and the fortieth hours it fell from its normal level of 300 mgm. per 100 cc. to less than 10 mgm. At the end of 72 hours the curve began to rise and was back to normal at the end of the fifth day. It is of interest that the fall in prothrombin began sooner than the fall in fibrinogen, and was slower in returning to normal. It would appear that here the prothrombin balance is not so easily maintained as is the balance of fibrinogen. It has been customary to assume that the prolonged bleeding time seen in such a case is due entirely to a deficit in fibrinogen. Our experiments, however, show that bleeding time begins to lengthen when the prothrombin has fallen markedly, at which time the plasma fibrin level may still be nearly normal. In two cases of liver necrosis produced by phosphorus poisoning, the prothrombin fell to less than 5 per cent before death. The fall in prothrombin associated with liver necrosis and its return to normal paralleling liver regeneration suggests that prothrombin formation is dependent upon liver function.

SUMMARY

A method is outlined for the accurate titration of prothrombin in plasma.

Normal dog plasma contains sufficient prothrombin to form more than 200 times the concentration of thrombin necessary to clot fibrinogen within a few seconds. When blood or recalcified oxalated plasma contains little thromboplastin it clots slowly, and much of this prothrombin remains unutilized. But in blood which contains large amounts of thromboplastin, and hence clots rapidly, the prothrombin is soon depleted by conversion to thrombin.

The plasma prothrombin level varies slightly in different dogs, but is remarkably constant in a given animal, even with repeated large hemorrhages or with drastic changes in diet.

A marked hypoprothrombinemia is produced by acute chloroform poisoning. The fall in prothrombin precedes the fall in fibrinogen and it returns to normal somewhat more slowly. Phosphorus poisoning also produces a profound fall in both prothrombin and fibrinogen.

In cases of severe distemper and of sterile turpentine abscess, the prothrombin level remains normal in contrast to the marked elevation of plasma fibrin.

Prothrombin is quantitatively normal in plasmas rendered spontaneously incoagulable by the intravenous administration of peptone, or by the *in vitro* addition of heparin. Incoagulable India ink plasma shows but slight reduction.

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THE PRODUCTION OF CHLORIDE-FREE SOLUTIONS BY THE ACTION OF THE INTESTINAL EPITHELIUM

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The ability of living systems to perform osmotic work is generally recognized. The mechanisms by which any such processes occur have not, however, been elucidated. The kidney is able to cause chloride to move from the glomerular filtrate into the plasma until the chloride concentration in the tubule fluid or the urine is virtually zero. In moving the last portions of the chloride the kidney cells perform osmotic work, the net result of which is to transport ions from one concentration to another at least a hundred times as great. Such a process is difficult of analysis when one has to deal with microscopic structures, and very small amounts of material for chemical analysis, among other difficulties. Burns and Visscher (1934) showed that a comparable phenomenon could be made to occur in the small intestine of the dog under suitable circumstances. They showed that when salts of relatively indiffusible anions such as sulphate, phosphate or citrate are placed in the small intestine along with sodium chloride, the chloride ion is removed to the blood against its concentration gradient. There was thus made available a convenient preparation for the study of ion transport against concentration gradients.

A further study of the conditions under which chloride impoverishment of physiological fluids occurs has been carried out by this method. The limit to which chloride-removal will go has been investigated. The changes in concentration of the nondiffusible anion in the system and the total ion exchange has been measured and the influence of substituting one monovalent cation (K) for another (Na) on the process has been observed.

METHODS. Observations were made on healthy adult dogs (and in a few instances on cats) anesthetized with amytal injected intra-peritoneally, 50 to 60 mgm. per kgm. The lowest 18 inches of the small intestine was reached through a midline incision, cannulated and flushed carefully but thoroughly with five liters of isotonic NaCl at body temperature. When the washings were clear each glass cannula was substituted for by a 1 inch length of heavy walled rubber pressure tubing with lumen occluded by a stem of glass rod. The fluids to be studied could thus be introduced by

removing the glass stopper and injecting directly from the glass nipple of a large syringe engaged in the lumen of the pressure tubing. Samples of fluid were removed from the central region of the loop at suitable times by means of a dry syringe and needle, the latter passed through the wall of the intestine. Great care was exercised in handling the gut as traumatization alters its behavior with respect to absorption. The blood flow was not interfered with in any way. The animal was kept warm by covering with dry cloths. These details are enumerated at the risk of repeating what might appear to be obvious precautions, because in our experience it is easy to fail to observe these details strictly enough.

As a standard procedure 50 cc. of a solution containing one-half isotonic quantities each of NaCl and Na_2SO_4, were placed in the lumen of the loop. It was found to be important to have the loop long enough so that the quantity of fluid introduced did not necessitate an intra-intestinal pressure greater than 5 cm. H_2O. Pressures large enough to interfere with capillary circulation were strictly avoided.

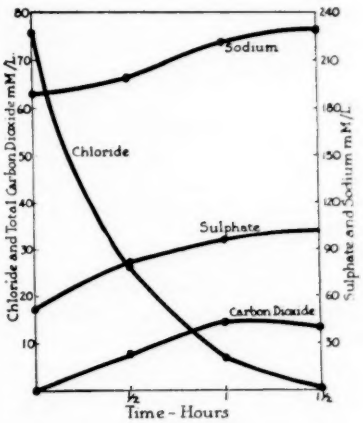

Fig. 1. Concentration changes in solution of sodium sulphate and chloride, each half isotonic, in a loop of lower ileum.

The chemical analyses were performed on samples of the intestinal fluid filtered immediately after withdrawal (except in the case of carbon dioxide analysis, in which case samples were not exposed to air). Chloride was determined by the method of Van Slyke (1923), sodium by that of Barber and Kolthoff (1928), sulphate by the benzidine method as detailed by Visscher and Smith (1935), carbon dioxide by the method of Van Slyke and Neill (1924), and potassium by that of Breh and Gaebler (1930).

Results. More than a hundred experiments have been performed in which the rate of change in concentration of various ions in the intestinal fluid in the presence of an indiffusible anion has been observed. Figure 1 shows an example of the changes in concentration of the several constituents studied in the typical case. As the chloride diminishes to 0.4 millimol (while the blood plasma chloride was approximately 100 millimols), the sodium content increases almost parallel with the concentration of the sulphate. The latter, being present originally in approximately half isotonic strength, is concentrated with the removal of water when the more

diffusible ions enter the blood. At the time when the chloride reaches very low figures the solution is approximately isotonic with respect to sodium sulphate. The sulphate ion in some experiments was apparently not absorbed at all. In other cases 50 per cent of the total sulphate was absorbed from the normal intestine. As will be pointed out in a subsequent report, the sulphate impermeability of the intestine is greatly affected by poisons. In an individual case the following net changes in ion content occurred. The loop of gut originally contained 50 cc. of fluid. Thirty cubic centimeters were absorbed during 1½ hours. Calculating from the analytical figures, the following changes took place in total movement of ions.

	ORIGINALLY PRESENT	FINAL CONTENT	NET CHANGE
	m Equiv.	*m Equiv.*	*m Equiv.*
Cl	3.84	0.20	−3.64
Na	9.52	4.63	−4.89
SO_4	5.68	3.88	−1.80
CO_2	0.01	0.31	+0.30
K	0.00	0.15	+0.15
NH_3	0.00	0.10	+0.10

It will be obvious that the total movement of chloride and sulphate is almost but not quite balanced by the migration of sodium. The total

TABLE 1

TIME	CHLORIDE IN MILLIGRAMS PER CENT												
hrs.													
0	248.2	301.1	258.0	271.1	295.0	306.8	270.5	272.2	272.2	280.0	296.0	270.4	258.0
½	49.9	55.0	190.7	132.8	84.6	280.4	220.4	186.1	153.0	73.9	230.0	62.2	123.8
1	21.9	23.2	139.2	46.6	24.8	198.8	165.0	34.4	122.3	16.3	125.0	12.5	23.5
1½	8.6	6.9	32.1	14.7	8.5	83.9	60.0	3.9	66.9	2.7	8.5	3.9	1.56

movement of anions and cations balances within the limits of experimental error and consequently one can rather safely conclude that the ions present in any great concentration have been accounted for. It is not certain, however, that other ions do not move, nor that the full influx of carbon dioxide or ammonia is as little as is left at the end of the period of absorption. One measures only the net changes and the possibility of moving in and out again is not taken into account.

The movement of chloride out of the intestine is sometimes so great as to make the remaining fluid virtually chloride-free. In the example shown in figure 1 the final chloride concentration was 0.37 mM per liter. The blood plasma contained, at the same time, approximately 100 mM per liter. Thus the final concentration was less than one-half of one per cent of the

blood value. Such extensive chloride improverishment does not occur in every instance when sodium sulphate and sodium chloride mixtures are placed in the intestine. A representative sampling of experimental results obtained in a series of observations on different animals is shown in table 1. The chloride level in the blood plasma was not determined in each of these experiments but in a large number of observations we have found that the plasma chloride does not deviate greatly from 350 mgm. per cent. The final chloride concentrations indicated in the table can be compared with that figure. It will then be seen that at $1\frac{1}{2}$ hour the fluid in the intestine may show chloride levels ranging from 0.5 per cent to 25 per cent of the plasma level. The most usual figure at the $1\frac{1}{2}$ hour period is 3 per cent of the plasma level, which implies that chloride is moved in the average case against a thirtyfold and in the exceptional case against a two-hundredfold concentration ratio. It was pointed out by Burns and Visscher (1934) that concentration ratios of that magnitude could not be set up by a Donnan equilibrium involving sodium chloride and sodium sulphate in which the sulphate is the indiffusible ion.

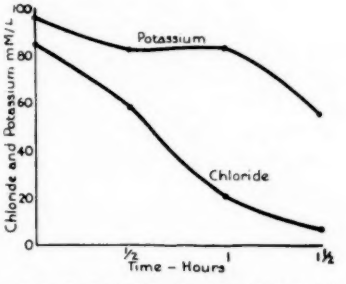

Fig. 2. Chloride impoverishment in solution of potassium sulphate and chloride, each half isotonic, in the lower ileum.

Newer data we have obtained permit a better calculation of the possible rôle of the Donnan effect. In a typical experiment the blood plasma analyses showed: chloride 111.8 m.M., sodium 149.1 m.M. per kgm. water. The initial concentrations in the intestinal fluid were: chloride 76.1 m.M., and sodium 200.6 m.M. The final concentrations after $1\frac{1}{2}$ hours were: chloride 1.8 m.M., and sodium 229.0 m.M. If one assumes that at equilibrium no other factors are involved $(Na^+)_0 \times (Cl^-)_0 = (Na^+)_i \times (Cl^-)_i$, the product should be $111.8 \times 149.1 = 16669$, calculating from the plasma figures. The initial ion product in the intestine was $76.1 \times 200.6 = 15266$, a figure smaller than the plasma factor, indicating that if the Donnan effect alone were involved there should have been movement out of the plasma instead of the opposite. Actually the ion product after the $1\frac{1}{2}$ hours in the intestine was $1.8 \times 229.0 = 412$. Obviously since the ion products were deviating from equality a simple membrane equilibrium effect involving only sulphate as the indiffusible ion and sodium and chloride as the mobile ones cannot account for the chloride movement. More detailed considerations of the driving forces will be presented in other communications.

The question of the rôle of the cation in the process observed has been

investigated to the extent of determining the effect of replacing all of the sodium by potassium. In the experiment shown in figure 2, potassium chloride and potassium sulphate, each half isotonic, were placed in the intestine and the changes in concentration of each noted. It will be seen that aside from the fact that potassium tends to leave the fluid at a more rapid rate than does sodium, the results are no different. The chloride level falls rapidly and to a final figure comparable with those obtained in experiments using the sodium salts. The decline in potassium is probably due to replacement by sodium from the plasma in view of the very high concentration gradient for potassium from intestine to plasma. The cation sodium apparently does not play a specific rôle in the chloride removal.

SUMMARY AND CONCLUSIONS

1. In the presence of sulphate ions chloride is almost completely removed from solutions placed in the lower ileum. The concentration of chloride may fall to as low as 0.5 per cent of the blood level.

2. There is a concentration of sodium sulphate in the intestine. The percentage of sodium increases because a larger proportion of the osmotic pressure is due to sodium ions when the anion is bivalent than when it is univalent.

3. The normal intestine is highly impermeable to the sulphate ion, resulting in the retention of fluid under the circumstances of these experiments.

4. The movement observed is not produced by a simple Donnan equilibrium in which the sulphate is the indiffusible ion.

5. Substituting potassium for sodium salts in the intestine does not significantly alter the movement of chloride.

6. The occurrence of a mechanism for chloride movement against concentration forces in the intestine provides a convenient preparation in which to study such processes in the nearly intact mammal. It should be possible to elucidate the energetics of secretory processes in which osmotic work is done by the study of this mechanism.

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THE INFLUENCE OF VARIOUS POISONS ON THE MOVEMENT OF CHLORIDE AGAINST CONCENTRATION GRADIENTS FROM INTESTINE TO PLASMA

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By studying conditions altering the process of chloride-impoverishment by the small intestine described in previous communications it was thought that light might be thrown on the mechanism of the process itself. This report deals with the observed effects of arsenic, cyanide, hydrogen sulphide, fluoride and mercuric chloride upon this activity of the intestine. A preliminary note on the subject has been published (Visscher and Ingraham, 1935). Deductions are drawn from these observations concerning some aspects of the physicochemical mechanism by which chloride-impoverishment occurs. Further studies are now in progress which deal with the sources of energy for the osmotic work performed in the transport of chloride against concentration gradients.

METHODS. The preparation of the segment of small intestine (terminal ileum) was described by Ingraham and Visscher (1936), as were the methods of chemical analysis. In studying the effects of poisons three methods have been employed. In the first the chloride absorbing power of a given preparation was tested first before employing the poison to be studied, then the whole process was repeated with the addition of the test substance. In the second the poison was employed in the first trial and the recovery from it observed in the next period, while in the third method a control was run first, then the effect of the poison observed, and finally another control period was studied. The susceptibility of the gut mucosa to some of the poisons used prevented successful experiments in some cases with the second and third methods, and the deleterious effect of too much handling ordinarily interferes with more than three hours of observation on any one preparation. Controls to show the reproducibility of results in successive runs showed that almost without exception chloride removal was less complete in a second run than in the first and always so in the third. The fact that the preparation deteriorates thus spontaneously has led to a further control in most cases, that is, the simultaneous study of two adjacent loops, small enough and near enough to the end of the ileum to

obviate the difficulty observed by Burns and Visscher (1934) that the upper segments of the small bowel behave differently from the lower portions. The results reported are based upon concordant observations by the several methods.

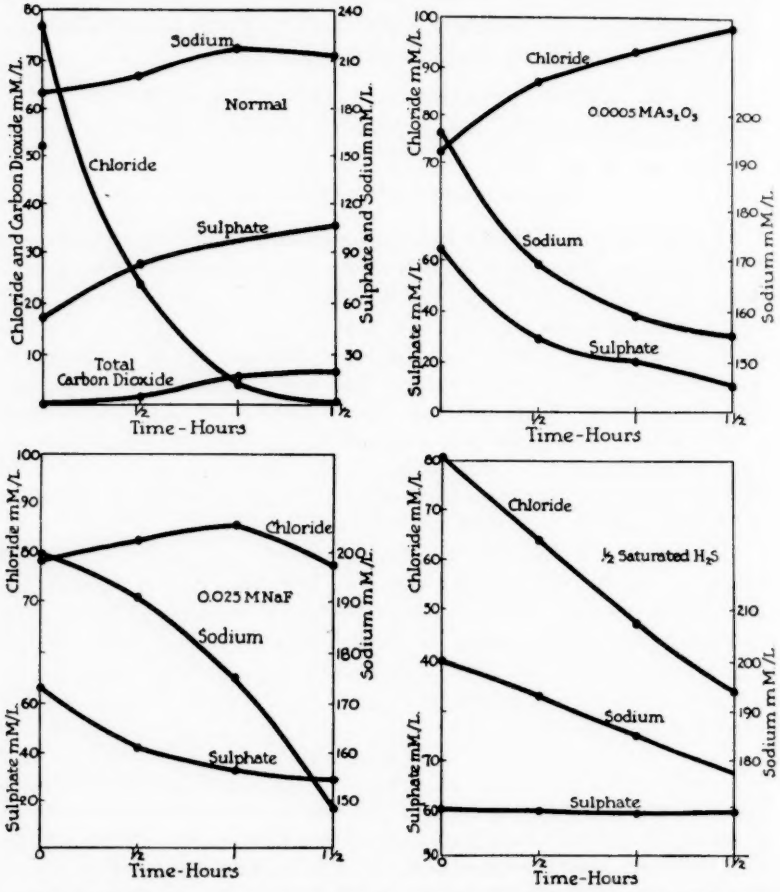

Fig. 1. Effect of various poisons on chloride impoverishment and related ionic concentration changes in isotonic mixtures of sodium sulphate and sodium chloride in the lower ileum.

RESULTS. In figure 1 are presented the observations in typical experiments in which chloride, sulphate and sodium were determined in cases where the intestine was poisoned by 0.0005M sodium arsenite, 0.025M

sodium fluoride and by half saturated solutions of hydrogen sulphide, in comparison with the normal control. These particular results were obtained by the second type of experimental procedure, but they are in qualitative agreement with observations by the other methods.

In contrast with the rise in concentration of sulphate and sodium coincident with the removal of chloride in the normal case over the course of time, in the experiments with these poisons the chloride concentration either quickly rises so as to approach the blood level (about 100 mM but not shown in the graph), as in the cases of arsenite and fluoride, or falls much

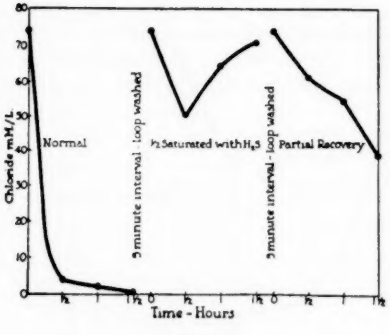

Fig. 2

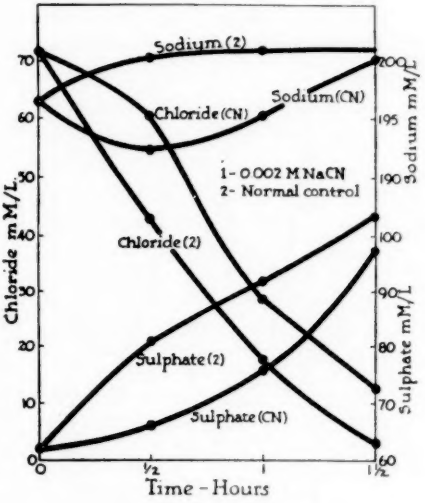

Fig. 3

Fig. 2. Poisoning and partial recovery of the chloride impoverishing mechanism by H_2S.

Fig. 3. Effect of cyanide on chloride impoverishment and related ionic concentration changes.

more slowly than normally, as in the case of hydrogen sulphide, and the sodium and sulphate concentrations tend to fall instead of to rise. In the cases of arsenite and fluoride poisoning the sulphate falls at such a rapid rate as to lead to the conclusion that the intestinal epithelium has become readily permeable to it, in contrast with the high degree of impermeability in the normal state.

The smaller effect of hydrogen sulphide in the concentration employed (half-saturation at atmospheric pressure) may be due to rapid absorption or destruction of the poison, but more pronounced effects were sometimes seen. In another experiment in which only chloride was determined,

shown in figure 2, the removal of chloride was rapid in the normal control period, while on testing with the same solution, but half saturated with hydrogen sulphide, the chloride level rose shortly to about the blood concentration and after washing and retesting with the unpoisoned solution a partial recovery was observed. On no occasion has complete recovery occurred after hydrogen sulphide, sodium fluoride or sodium arsenite in doses adequate to bring about reversal of chloride movement.

The effect of cyanide is somewhat different from that of the poisons previously discussed. Figure 3 presents the results of an experiment in which in the initial run sodium cyanide was introduced with the sulphate chloride mixture. In the second period the poison was omitted. The differences in the course of the concentraton changes in the two cases are

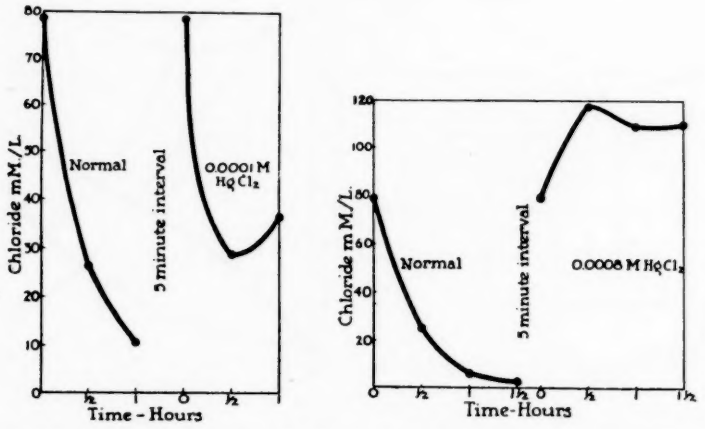

Fig. 4. Effect of concentration of $HgCl_2$ on the chloride impoverishing mechanism

exactly those predictable from a very evanescent poisoning of the same qualitative type seen with the other toxic agents. Cyanide behaves as does arsenite, only the effects of a single dose are not observable for longer than a half-hour. Larger doses could not be employed because they caused death of the dog following a severe respiratory storm. The positive effect of sodium cyanide is of interest because it, along with arsenite, is a rather selective poison of catalytic oxidation. Some of the other agents used are quite nonspecific poisons.

The effect of mercuric chloride, and in particular the graded effect in relation to concentration of the poison, is shown in figure 4 where the results of two experiments are presented. In the first a concentration of 0.0001 M mercuric chloride just perceptibly decreased the chloride-

impoverishing power of the intestine. In the second a concentration of 0.0008M entirely reversed the direction of chloride movement.

It seems apparent that these poisons abolish the capacity of the intestine to perform osmotic work upon the chloride ion in removing it from the intestine at very low concentrations to the plasma where its concentration may be two hundred times as great (Ingraham and Visscher, 1935). The fact that in every instance associated with the loss of this capacity there is a loss of the property of impermeability to the sulphate ion leads one to conclude that impermeability to sulphate is one of the conditions necessary for the occurrence of chloride movement against concentration gradients. It was shown by Burns and Visscher (1934) that phosphates and citrates were equivalent to sulphates in providing the conditions for chloride-impoverishment. It may therefore be rather safely deduced that impermeability to di- and polyvalent anions is the general fundamental condition necessary to the operation of other physicochemical processes incident to the performance of this type of osmotic work by the intestine. The impermeability of the membranes to these ions obviously results in the retention of water along with the salts. The prevention of water absorption due to the higher valence anions thus allows the decrease in chloride concentration when that ion leaves the intestine. Normally water and salt absorption nearly parallel one another and it is only when the water is held that the concentration of the ions which are absorbed can be markedly reduced.

Although it had not previously been shown in the case of sulphate impermeability it is not surprising that the intestine should become more permeable in the presence of the poisons employed. Cohnheim (1898–99) showed that fluoride altered the behavior of the intestine toward glucose and sodium chloride. He believed that the intestine was unidirectionally permeable to chloride, and that fluoride abolished the selectiveness of the permeability. Burns and Visscher (1934) found however (see their fig. 1) that sodium and chloride readily entered distilled water in the intestine and that equilibrium was reached at concentrations slightly above their plasma levels. Only when the fluid in the intestine contained di- or trivalent anions was chloride moved preferentially into the blood. The effect of poisons upon the process under consideration is not one of increasing permeability to chloride, because there is ample evidence of a high degree of permeability to it normally. The poisoning is seen to consist rather, at least in part, in an increase in the permeability of the intestine to anions of higher valence.

There is evidence, which will be presented in a later paper, that the poisons under consideration also affect certain specific processes, especially the formation of ammonia, by the intestine. Exactly what part in the reversal in chloride movement brought about by poisons, is due to the loss

of sulphate impermeability, and what proportion to the disturbance of specific metabolic processes, can be discussed better in connection with the observations upon the latter.

The rôle of the di- and polyvalent ions in this type of process is probably of general significance. In the secretion of urine, chloride reabsorption is one of the most important processes. Here one finds large concentrations of phosphate and smaller quantities of citrate and sulphate as normal occurrences. When the kidney is poisoned by cyanide (Starling and Verney, 1925) the sulphate is no longer concentrated and the chloride goes to the blood level. Such poisoned kidneys secrete a urine which is approximately an ultrafiltrate of plasma. The similarities in the two cases bespeak a common mechanism for chloride impoverishment in the kidney and in the intestine when di- or polyvalent anions are present. The membrane impermeability to these ions is apparently a first requisite to the chloride movement.

It may be pertinent to point out that if the chloride movement in the case of the kidney constitutes a secretory process, it must then also be so looked upon in the intestine. One would then have to speak of secretory absorption. It is unfortunate that a simple term is not available to cover the case. Chloride removal or impoverishment are expressions which have been used in these papers, and are probably satisfactory, but the point should be understood that these terms are synonymous with what has ordinarily been called secretory absorption, since osmotic work is being done. As these phenomena are being elucidated from the point of view of their mechanisms the significance of the term secretion in connection with them will become clearer, or one may substitute for it more definitely descriptive names of the fundamental processes involved.

SUMMARY AND CONCLUSIONS

1. The chloride-impoverishment in the intestine occurring in the presence of sulphate ions is abolished by adequate concentrations of sodium arsenite, sodium fluoride, hydrogen sulphide, mercuric chloride and sodium cyanide.

2. In the presence of these poisons the chloride moves into the intestine under circumstances under which it would otherwise move out.

3. The increase in concentration of sodium and sulphate ion in the intestine, which normally occurs under the conditions described, is abolished or reversed under the influence of the poisons enumerated. It may be inferred that the intestine which is normally relatively impermeable to the sulphate ions becomes permeable to it in the presence of these substances.

4. Impermeability to divalent or polyvalent anions is apparently one of

the fundamental conditions necessary for the performance of osmotic work on the chloride ion by the intestinal epithelium.

5. The rôle of the di- and polyvalent anions is probably of general significance in other processes of chloride-impoverishment since in the kidney conditions are normally present comparable to those in the intestine in these experiments.

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OBSERVATIONS ON THE PATH TAKEN BY THE PAIN FIBERS FROM THE HEART

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Considerable attention has been paid in recent years to the pathway taken by pain fibers from the heart to the central nervous system (Heinbecker, 1933; White, Garrey and Atkins, 1933; Moore and Singleton, 1935). Several methods have been used in studying this problem. One method has consisted of analyzing the nature of the afferent fibers in various nerves connecting with the heart, according to their size and myelination and of correlating these findings with their electrical response to noxious stimuli as compared with the type of response obtained from somatic pain fibers. While the possible pain fibers from the heart can be mapped out in this way, the actual pain fibers cannot be so located because the fibers so defined may come from regions other than the heart, or may transmit sensations other than pain.

Another method has consisted of interrupting various peripheral nerve tracts in patients with angina pectoris either by resection, local anesthesia or chemical destruction. The literature on this subject is extensive, the nerve tracts recommended to be interrupted are many and the results not entirely in accord (cf. White, Garrey and Atkins, 1933, for recent review). In consequence of this disparity attempts have recently been made to correlate the clinical studies by observations on animals. Several workers including Singer (1926), Sutton and Lueth (1930) and Katz, Mayne and Weinstein (1935) have shown that in the dog the pain fibers are concentrated in the plexus of nerves shown by Woollard (1926) to surround the coronary vessels. Katz, Mayne and Weinstein (1935) showed that the pain tracts are limited to the region of the coronary vessels, the rest of the epi- and myocardium being seemingly free of pain fibers and endings. White, Garrey and Atkins (1933) found that in the dog removal of the sympathetic chain from the first to the fourth thoracic segments or section of the upper five dorsal roots of these segments could abolish the pain sensations coming from the heart. The efficacy of severing the sympathetic chain has been confirmed by Moore and Singleton (1935); they severed the chain as low as the seventh rib. White, Garrey and Atkins (1935) reported that section of the vagi or removal of the stellate ganglia

was without such effect. Moore and Singleton (1935) found that section of the vagi or cervical sympathetic chain was without such effect. They found that the pain impulses from the heart spread bilaterally and somewhat symmetrically to the two sympathetic chains.

It occurred to one of us (W. M.) that the pain fibers might be interrupted in their course from the heart to the upper thoracic sympathetic chain. This procedure, it seemed to us, would offer certain advantages physiologically. By interrupting the pain fibers nearer the heart it would be possible to avoid severing other fibers which join them further away. Thus the denervation of the lungs would be less complete if not entirely avoided. There would be no interference with fibers, both efferent and afferent, joining the upper thoracic sympathetic chain and the abdominal viscera and the head, neck and arms. By going nearer to the heart it might be possible to sever pain fibers which might not enter the cord by the upper thoracic dorsal roots and might not even go to the stellate ganglion but pass directly into the cervical sympathetic chain to enter the central nervous system in the cervical region or by cranial nerves (such as the fifth or tenth).

A careful dissection of the nerves leaving the thoracic sympathetic chain revealed a large number of fibers coursing medially into the posterior mediastinum and terminating in the deep cardiac plexus. These fibers connect with the stellate ganglion and the thoracic ganglia as low as the sixth. Some fibers from the vagi and cervical sympathetic chain are also to be found here.

An attempt was made in the present study to see if severing the cardiac fibers in the posterior mediastinum would abolish the appreciation of noxious stimulation of the coronary vessel nerve plexus of the heart.

Under ether anesthesia a pericardial fistula to the exterior was made by means of a glass tube after the method of Sutton and Lueth (1930). A ligature was passed under the anterior descending branch of the left coronary artery and its veins and the surrounding nerve plexus and the two ends brought out through the fistula. The animals were then permitted to come out from the anesthetic and after they had recovered fully, traction was applied to the ligatures and the affective response judged according to the criteria reported by us previously (Katz, Mayne and Weinstein, 1935). This procedure was followed in 15 dogs to serve as controls, after which the animals were sacrificed. In every one of these animals positive proof of an affective response was obtained.

In three further dogs this procedure was preceded by section of the nerve plexus in the posterior mediastinum. This was accomplished under ether anesthesia. The pleura reflected on the superior mediastinum opposite the esophagus was cut, the index finger was passed under the esophagus on either side, thus elevating it from its bed on the vertebral column. All

nerve fibers encountered were cut as the finger passed down from the level of the stellate ganglion to the arch of the aorta on the left and the azygos vein on the right. In addition on the left side a narrow strip of the posterior part of the aortic arch was freed from its surrounding tissue extending downward for about two centimeters to insure severing the fibers going to the fourth, fifth and sixth thoracic ganglia. After these animals had recovered fully from the operation and anesthesia, it was found that traction on the ligature around the coronary vessel nerve plexus gave no affective response although stimulation of somatic sensory nerve gave the usual response.

As a further check to rule out the possibility that the foregoing animals might have been unresponsive from the start two other dogs were prepared as in the control experiments without interfering with the nerves in the posterior mediastinum. After we found that they gave a positive response

TABLE 1

Table of results

NUMBER OF DOGS IN GROUP	AFFECTIVE RESPONSE FOLLOWING STIMULATION OF CORONARY NERVE PLEXUS			CHECK OF ANIMAL SENSIBILITY AFFECTIVE RESPONSE FOLLOWING STIMULATION OF SENSORY SOMATIC NERVE
	Posterior mediastinal nerves intact	After bilateral posterior mediastinal nerve resection	After "mock" operation in posterior mediastinum without severing nerves	
15	Positive			Positive
3		Negative		Positive
2	Positive	Negative		Positive
2	Positive		Positive	Positive

on traction of the ligature around the coronary vessel nerve plexus, they were reanesthetized and the nerve resection in the posterior mediastinum performed as described above. On recovering from the anesthesia and the operation these animals did not give any evidence of any effective response following traction on the ligature around the coronary vessel nerve plexus, although they were in excellent condition and responsive to somatic nerve stimulation.

As a final check to rule out the possibility that the double operation had rendered the animals unresponsive, two other animals were tested for their affective response following traction on the ligature around the coronary vessel nerve plexus without interfering with the nerves in the posterior mediastinum. These dogs gave positive affective responses to traction of the coronary ligature on recovering from their preparatory operation. The animals were then reanesthetized and subjected to the same type of operation as in the posterior mediastinum nerve resection operation except that the nerves were not harmed. After this "mock operation" the dog on

recovering from the anesthesia still gave positive affective responses to stimulation of the coronary vessel nerve plexus.

The results of these experiments are summarized in table 1 where it can be seen that severing the nerves from the heart located in the posterior mediastinum does abolish the affective response obtained from stimulation of the coronary vessel nerve plexus. There can thus be no doubt that the pain fibers from the heart do traverse the plexus of nerves in the posterior mediastinum. Of course these experiments do not rule out the possibility of other paths of stray pain fibers from the heart but they would lend support to the belief that by far the major portion are located in the posterior mediastinum. Further it follows that the number of pain fibers remaining after this resection—if there are any—is insufficient to permit the impulses set up by stimulating the coronary vessel nerve plexus to exceed the threshold for pain appreciation in these animals.

On the assumption that these results can be applied to man, work is in progress to determine the effect of this operation on the relief of pain in patients with angina pectoris.

The possibility remains to be investigated whether or not this nerve plexus in the posterior mediastinum conveys pain fibers from the aorta. The further problem also remains whether or not these paths contain many of the vasoconstrictor fibers, which recent work from this laboratory suggests reach the heart via the sympathetic innervation (Katz, Jochim, Weinstein and Bohning).

SUMMARY

1. Evidence is presented to show that cardiac pain fibers traverse the nerve plexus located in the posterior mediastinum which connects the deep cardiac plexus with the thoracic sympathetic chain from the sixth up to the first thoracic segment and possibly also with the cervical sympathetic chain.

2. Severance of the fibers in the posterior mediastinum was found to abolish the affective response obtained on stimulating the nerve plexus surrounding the coronary blood vessels.

3. The feasibility and possible physiological advantages of this operation in patients with angina pectoris are discussed.

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SOME EFFECTS OF LAMINAR THERMOCOAGULATION UPON THE LOCAL ACTION POTENTIALS OF THE CEREBRAL CORTEX OF THE MONKEY[1]

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We wish to report briefly some of the results of laminar thermocoagulation upon the action potentials of the cerebral cortex of the monkey (macacus) under even "Dial" anesthesia.[2]

The records were obtained with a cathode ray oscillograph and a two-stage D.C. amplifier, grounded between stages to short out capacities, which otherwise prevent amplification of high frequencies. The small silver-silver chloride electrodes used—both necessarily alive—were of a type known to give a very small and stable E.M.F. in the presence of chloride ions. The electrodes were placed upon the cortex 3 mm. apart. This "bipolar" method was preferred to the usual "monopolar" method, because the latter results in a composite picture of the action potentials, originating anywhere in the body between the electrodes, whereas the bipolar method, as used here, restricts the potential differences to those arising in the area under investigation. After thermocoagulation, involving in each instance an area of 5×7 mm., the electrodes were replaced exactly in their original positions, well within the area coagulated.

The *results* to be reported here are the following:

1. Thermocoagulation at 80°C. for 5 seconds, killing the entire thickness of the cortex, immediately and permanently abolishes the characteristic action potentials normally found in the particular cortical area under the conditions of these experiments.

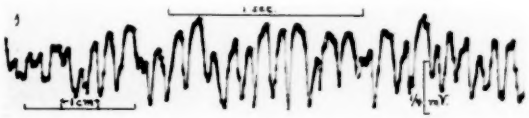
2. Laminar thermocoagulation at 70°C. for from 4 to $4\frac{1}{2}$ seconds, killing the outer four layers, reduces the local action potentials permanently and almost completely.

3. Laminar thermocoagulation at 70°C. for 3 seconds, killing the outer three layers, reduces the local action potentials markedly with little or no evidence of any return to their original size and form. This reduction is much less than that after laminar thermocoagulation of the outer four layers.

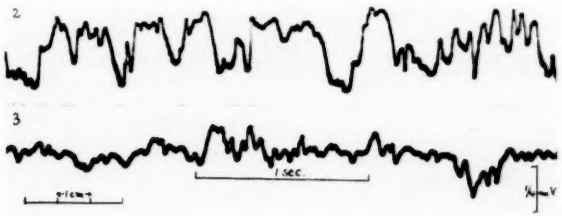
[1] This investigation was aided by a grant from the Research Funds of the Yale University School of Medicine.

[2] The Dial was kindly put at our disposal by the Ciba Company.

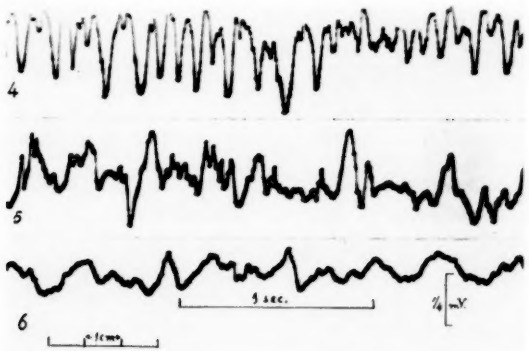
4. Laminar thermocoagulation at 65°C. for 3 seconds, killing the outer two layers of the cortex, results in a definite reduction in the local action



Record 1. Spontaneous action potentials from "normal" precentral "motor" face region.



Records 2 and 3. Spontaneous action potentials obtainable from sensory face region. 2 is taken from "normal" cortex, 3 is taken after local thermocoagulation of 3 outer layers of part of this cortex.



Records 4, 5 and 6 are recorded from same region as record 1: 4, ten minutes after thermocoagulation of 3 outer layers of sensory face area; 5, twenty-five minutes after this thermocoagulation. 6 is taken four minutes after laminar thermocoagulation (of 3 outer layers) of the motor face area itself.

potentials, from which there is some return, though they do not regain their initial size and shape at the end of one hour.

5. Laminar thermocoagulation of the outer three layers, at 70°C. for 3 seconds, of one area (5 × 7 mm.) in the precentral, postcentral or frontal

region produces changes in the local action potentials within each of the other two regions. Without thermocoagulation, even in the course of several hours, no such changes occur.

Taken together, the findings sub 1, 2, 3 and 4 show *1*, that these local action potentials originate in the cortical area under investigation, and *2*, that the action potentials remaining after any thermocoagulation arise in the remaining layers of the cortex.

The finding sub 5 demonstrates the existence of a functional interrelation among these three regions. Whether a functional interrelation obtains with respect to other regions of the cortex is still under investigation.

The accompanying figure illustrates findings 3 and 5. The speed of paper, the amplification and the reduction used in reproducing the original records are all indicated on the illustrations.

Records 1 and 2 show the difference in the action potentials in the *pre*central and *post*central "face" regions of a cortex intact.

Records 2 and 3 are taken from the *post*central "face" region, before and after laminar thermocoagulation of its outer three layers, and demonstrate the marked changes, especially the reduction, which occur after such destruction.

Records 4 and 5 are from the *pre*central "face" region, the electrodes being located exactly as in record 1. They illustrate the progressive diminution of the action potential obtainable from this region, ten and twenty-five minutes after the laminar thermocoagulation of the *post*central "face" region.

Record 6, still from the *pre*central "face" region, shows the ultimate reduction of its residual action potentials (after records 4 and 5 were taken) four minutes after thermocoagulation of its own outer three layers.

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EFFECT OF ACETYLCHOLINE ON THE BLOODFLOW THROUGH THE STOMACH AND LEGS OF THE RAT[1]

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The newer knowledge about the liberation of an acetylcholine-like substance when nerve endings and synapses are stimulated, as propounded by Loewi (1), Dale (3), Babkin, (4), and their collaborators, has opened a new chapter in the physiology of organ-relations. Hunt (5) and Dale (6) described the tremendous vasodilator properties of acetylcholine and its vagus-like effects on heart, intestinal motility, etc. In one of his papers Hunt (5) mentions one experiment in which the blood vessels of the intestine were contracted, while all other organs tested showed vasodilatation, when they were perfused with dilute solutions of acetylcholine. Hirose (7) reported similar findings when larger doses of acetylcholine were used. Recently, Feldberg and Dale (8) found that stimulation of the gastric vagus-nerves definitely increased an acetylcholine-like substance in the venous blood from the stomach.

As yet nothing is known about the effect of acetylcholine upon the vascular bed of the stomach.

In the stomachs of patients with peptic ulcer, an acetylcholine-like substance may be liberated either in larger amounts or may be present more consistently than in normal persons. Evidence for this may be seen in the greater and more consistent motility of the stomachs of ulcer patients (9). Since the presence of an acetylcholine-like substance might have an important effect on the blood supply to the tissues of the stomach, the authors studied the effects of acetylcholine on the bloodflow through the surviving stomach of the rat. As controls, the hind legs of the rat and the ear and heart of rabbits were studied.

METHODS. Albino rats and big black and white rats were used. They were sacrificed, and immediately afterwards the abdomen and thorax were opened and a cannula inserted into the aorta just below the diaphragm. Perfusion with oxygenated Locke's solution was started immediately, so that

[1] A preliminary report of this work was presented at the 47th Annual Meeting of the American Physiological Society, 1935.

Aided by the Louis L. Cohen Fund.

the stomach was without circulation for only a short time (5–10 min.). Then the aorta was ligated below the celiac axis and cut. All other branches of the aorta except the celiac axis were ligated. The hepatic artery was tied and cut. The splenic artery was ligated as near as possible to the spleen and the latter removed. The lesser and major omentum were tied and cut, but proper care was taken not to place the ligatures too near to the stomach so as not to ligate the main branches of the gastric arteries. The portal vein was cut, but not ligated. Arterial bleeders were detected easily by their spurting and ligated. Cannulas were inserted into esophagus and duodenum, and the stomach was thoroughly washed with Locke's solution. The stomach was placed on a flat block of paraffine and held in place by the three cannulas which were fixed onto the paraffine. The preparation was put into a moist chamber. The pressure of the perfusion fluid was 100 cm. of water in most cases. The perfusion fluid from the portal vein ran into a beaker along a groove in the paraffine. Since the preparation survived longer at room temperature (23–26°C.) than at 38°C. and since the results were similar at both temperatures, most of the experiments presented were done at room temperature. Constant control values were obtained and the effects of drugs (10) were constant, even when a slight edema was present. A similar technique for perfusion was employed for the preparation of rats' legs and rabbits' ears. Most preparations were viable for 2 to 2½ hours.

Since acetylcholine in Locke's solution partly disintegrated even at room temperature, all solutions were made freshly before use.[2] Only such experiments are reported here in which constant controls were obtained with Locke's solution. Hemoglobin Ringer, (11, 12) was tried, but had to be discarded because our preparation of it "killed" the stomach. Each control period lasted 2 to 5 (usually 2) minutes and 4 to 10 control periods were taken before and after perfusion with drugs.

RESULTS. *A. Rats' stomachs and acetylcholine.* Forty-five tests on 23 rats' stomachs were made. In 44 of the tests acetylcholine produced a decrease in the rate of perfusion. In one test an increase was found. Results are considered significant when the difference is greater than ±10 per cent. Changes amounting to less than 10 per cent occurred in 11 tests; but most of these occurred with higher dilutions of the drugs.

Acetylcholine 1:2,000,000 lowered the perfusion rate by 10 per cent, which change is insignificant or of borderline significance. The average vasoconstrictor effect of the other dilutions varies between −14 and −22 per cent, with no clear direct relation between concentration of acetylcholine and vasoconstrictor effect. It is evident that every rat's stomach is different in its quantitative response to acetylcholine, and that it is somewhat open to objection to consider averages of results from different

[2] We are obliged to Hoffman-La Roche for a liberal supply of acetylcholine.

stomachs. It may be stated, however, that in 44 out of 45 tests on 23 stomachs a decrease in flow was found, varying between −3 and −44 per cent with a total average of −17 per cent.

B. Perfusion of rats' stomachs with acetylcholine, atropine, eserine and other drugs. These tests were made to determine whether our test object, the rat's stomach, possessed the known reactions to acetylcholine after eserinization and atropinization. The sensitizing effect of eserine (probably by inhibition of tissue esterase) and the antagonistic effect of atropine on acetylcholine were confirmed. Histamine had no clear cut effect on the blood vessels of the stomach. Lim, Necheles and Ni (13) working with dogs' stomachs observed vasodilatation after the injection of histamine into the celiac artery (oncometry). Sodium nitrite elicited slight vasodilatation.

C. Perfusion of rats' legs, etc. Sixteen tests were performed on the hindlegs of six rats. Acetylcholine in dilutions of 1:50,000 to 1:2,000,000

TABLE 1

Perfusion of rats' stomach

NO. OF EXPTS.	DILUTION	AVERAGE ± PER CENT CHANGE	MAXIMUM AND MINIMUM PER CENT CHANGES
7	1:50,000*	−18	−5 to −29
8	1:100,000†	−22	−11 to −32
5	1:200,000	−19	−14 to −28
5	1:500,000	−14	−4 to −23
11	1:1,000,000	−19	−4 to −44
8	1:2,000,000	−10	−3 to −18

* Contains one test 1:25,000.

† Contains one test 1:125,000.

produced vasodilatation in every one of 5 tests. After atropine, acetylcholine did not produce a change from the control values except in one case. One rabbit's (albino) ear was perfused. A concentration of acetylcholine of 1:100,000 produced an increase of flow of +37 per cent and +36 per cent respectively. One heart of a rabbit (albino) was perfused, using Langendorf's method, the flow through the coronary system being measured. In one test, a concentration of acetylcholine of 1:500,000 increased the rate of perfusion from 6 to 14 cc. per minute and from 6 to 10 cc. per minute in another (+133 and +67 per cent respectively), the heart rate being decreased from 115 per minute to 72 and 44 respectively. The flow then returned to 6 cc. per minute and the heart rate to 108 beats per minute. This experiment was done merely to ascertain if our preparation of acetylcholine manifested the well known pharmacological properties of this drug. Our results confirm the work of other observers (6, 14, 15, 16) except that of Reid Hunt (17) who observed constriction of the blood vessels of the rabbit's ear.

DISCUSSION. Acetylcholine is recognized as an effective cardiac depressant and as a vasodilator. Moreover, it has a powerful motor effect on most parts of the gastro-intestinal tract. In the experiments presented above, the motility of the stomach was not recorded. But the stomach was closely observed during the experiments and only on very few occasions (1 to 2 times) was motility observed. The lack of motility may be due to damage to the motor system of the stomach by the perfusion, because the viviperfused stomach of a dog responds to acetylcholine with vigorous contractions. A series of experiments has just been concluded in this laboratory demonstrating that isolated gastric and mesenteric veins and capillaries are constricted by acetylcholine. The rats' stomachs yielded constant control results for hours and responded to various drugs with amazing constancy.

This work confirms the observation that acetylcholine has a vasodilator effect upon peripheral vascular areas, e.g., the hind legs of the rat, the rabbit's ear, and the coronary vessels of the heart. To our knowledge, no evidence has been published which demonstrates that acetylcholine has a vasoconstrictor action on the blood vessels of the stomach. In 44 tests on 23 rats such action was shown to be present. Vasodilatation occurred (+24 per cent), in only one test. We have found that in the vivi-perfused dog's stomach the higher dilutions of acetylcholine produce vasoconstriction; the greater concentrations, vasodilatation. In the stomach of rats, this reversal of action was found only once.

Since the stomachs of rat and man represent such essentially different structures, the above results cannot be considered as directly pertinent to an interpretation of the etiology of gastric ulcer in man.

SUMMARY

The stomach and hind legs of rats were perfused with Locke's solution to which various amounts of acetylcholine were added. In 45 tests on the stomach, vasoconstriction was obtained 44 times. A dilution of acetylcholine greater than 1:2,000,000 appeared to be without effect.

The hind legs of rats showed vasodilatation in 5 tests when perfused with acetylcholine. Atropine counteracted the vasoconstrictor effect of acetylcholine on the stomach in every case and the vasodilator effect on the hind legs in all tests but one.

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A MICROMANIPULATIVE STUDY ON THE MIGRATION OF BLOOD CELLS IN FROG CAPILLARIES

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This paper deals with the reaction of the blood cells in the capillaries of the frog's mesentery to localized irritation produced mechanically with microneedles or chemically by injecting acids with micropipettes.

The method consisted in exposing the intestinal loop of a frog and placing it on a specially constructed horseshoe-shaped chamber on the stage of the microscope (Zweifach, 1934). The arrangement permitted the use of microneedles for insertion into the mesentery from beneath. The upper surface of the mesentery was covered with a coverslip and the observations were all made with oil immersion lenses. A substage condenser with a working distance of 10 mm. was employed. The frogs used were Rana pipiens and Rana catesbiana during both summer and winter months. They were immobilized by severing the spinal cord between the first and second vertebrae with a hot needle.

When a preparation is properly mounted, blood flow in the capillaries is swift and steady. Erythrocytes are carried along in the center of the stream, while an occasional leucocyte tends to roll more slowly along the wall. A useful criterion for selecting capillaries is the degree of their distention. Capillaries, injured during the preparation, become rapidly dilated while normal vessels remain fairly constant in diameter and tend to increase in width only after an exposure of several hours to light.

A slight local irritation readily causes leucocytes to adhere to the endothelium. With continued irritation the erythrocytes become attached, followed frequently by their extravasation. The leucocytes extravasate far more readily than the erythrocytes and over a more extended region of the capillary wall beyond the actual site of irritation. More pronounced irritation, short of actual tearing the endothelium, results in the accumulation and packing of blood cells within the vessel to the extent of interrupting blood flow. Complete or partial recovery may take place after any of these stages.

EXPERIMENTAL RESULTS. 1. *Attachment of blood cells to the endothelial wall and to each other.* a. *Leucocytes.* A capillary in good tone was selected and a single endothelial cell in its wall, recognizable by the

position of its nucleus, was gently rubbed. A leucocyte adhered temporarily to the stimulated region. Repetition of the rubbing caused six leucocytes to adhere at and below (i.e., downstream) the site of stimulation. The more dilated the capillary the more readily would leucocytes adhere when the vessel was rubbed.

The leucocytes retain their adhesiveness for some time after they have come in contact with the mechanically irritated portion of the endothelial wall or to other leucocytes which were already adhering there. This was indicated by the fact that adhering leucocytes, when swept away by the force of the blood stream, tended to adhere again to the wall of the capillary beyond the irritated spot and also to post-capillaries, venules and even veins some distance away. With more intense irritation these dislodged leucocytes sometimes adhered to each other and formed aggregates of such size as to obstruct the flow of blood especially at points of branching or bending of vessels.

The adhesiveness does not persist. For example, after an aggregate has once formed (lasting 2 to 30 minutes according to the intensity of the stimulus) fresh leucocytes may fail to adhere to the aggregate which begins to fall apart. The leucocytes loosen from the wall and roll along the stream either singly or in small groups which eventually become completely separated.

A similar reaction was obtained by irritating with an acid. A droplet of 1 per cent acetic acid in olive oil, about twice the size of a red blood corpuscle, was injected close to but not touching the wall of a capillary. Within a few minutes eight leucocytes adhered to the inside of the endothelial wall adjacent to the droplet. Several minutes later two of the leucocytes became dislodged, three migrated through the wall while the remainder remained in position for over one hour. Many repetitions of this experiment gave similar results.

b. *Erythrocytes.* After a slight irritation erythrocytes make only momentary contacts. With greater irritation they become attached and will remain in position after the leucocytes have been swept away or have extravasated. These erythrocytes finally either become extravasated or drop back into the blood stream. With still more severe irritation, both leucocytes and erythrocytes become attached and extravasate at about the same time.

Leucocytes present a broad adhering surface to the wall whereas erythrocytes never have been seen to be attached at more than one small portion of their surface. Their entire surface may often be in contact with the endothelium but the attachment is only at one point. This can be shown by interrupting the blood flow, using the side of a microneedle to clamp the capillary beyond the region of attachment. The attached erythrocyte can then be seen to sway back and forth and exhibit plainly a one point of

actual attachment. When the clamp on the capillary is released the sudden return of the flow sometimes causes the attached erythrocyte to be stretched to a considerable length by the force of the stream. With a retardation of the current the cell resumes its original shape and the procedure may be repeated several times.

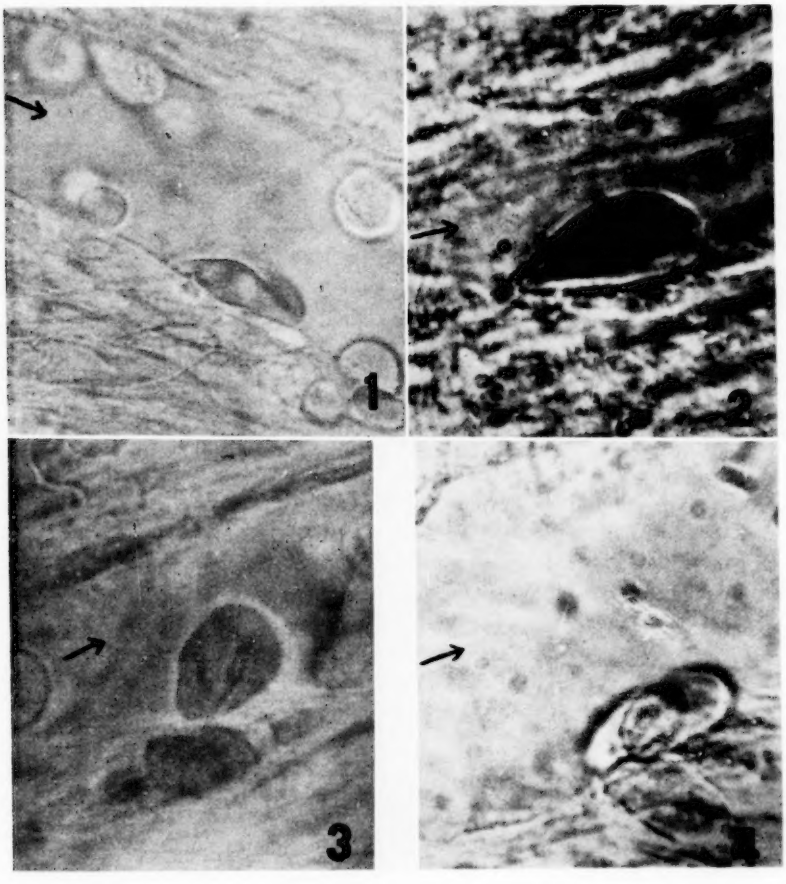
Figures 1 and 2 in the accompanying plate are from photographs of erythrocytes just after their attachment to the wall of the capillary. In Figure 1 the erythrocyte is seen with a small bulge of the extravasated portion visible on the outer wall of the capillary. This erythrocyte was observed to extravasate completely during which time the blood flow in the capillary continued uninterrupted.

Figure 2 shows an attached erythrocyte with the main part of its body being pulled upon by the force of the blood stream.

2. *Extravasation.* a. *Leucocytes.* The diapedesis of leucocytes may be regarded in two stages: first, their adhesion to the endothelial wall and second, their actual migration through the wall. The leucocyte migrates by the extension of a narrow pseudopod through an opening in the endothelial wall covered by the leucocyte. With the continued streaming of protoplasm into the pseudopodium the cell becomes deeply constricted while the nucleus, especially in the case of the larger leucocytes, generally passes through last. During the later period of the passage the rate becomes considerably more rapid as if the cell were then slipping through by pressure from behind. In all the cases observed, the site of the migration was at an appreciable distance from the nuclear swellings of the endothelium indicating that the passage occurs between the endothelial cells. No leucocytes were seen passing back into the blood stream from the surrounding tissues.

b. *Erythrocytes.* Erythrocytes, in contrast to leucocytes, appear to be passive during the entire period of their passage through the wall. A partially extravasated erythrocyte is shown in figure 3 of the accompanying plate. The considerable degree to which the erythrocytes become constricted is well shown. If the capillary contracts, the passage of those erythrocytes which are almost through the wall tends to be accelerated. Erythrocytes which have only begun to pass through may be forced back and set free in the blood stream.

Figure 4 shows a completely extravasated erythrocyte with a second erythrocyte just beginning to follow it at the same site. This indicates that the opening in the wall persists for some time. Such a persistence may also occur after the diapedesis of a leucocyte since several erythrocytes, one after the other, have been observed to pass through the same path used by leucocytes. This tends to occur in capillaries which have dilated after severe irritation. In such cases the first erythrocyte to pass through is usually only slightly constricted, while the following erythrocytes be-



Photographs of blood capillaries in frog's mesentery showing extravasation of erythrocytes. Arrows indicate direction of blood flow.

Fig. 1. Dilated capillary with cells attached to wall. Two erythrocytes (on lower side) are extravasating. The middle one (in best focus) has just begun and shows small bulging portion in interstitial tissue outside wall. This cell eventually was completely extruded.

Leucocytes (on upper side) two, rolling along and one, adhering and partly stretched by force of blood stream.

Fig. 2. Erythrocyte caught on capillary wall and being dragged upon and wrinkled by force of blood stream.

Fig. 3. Dumb-bell shape of partly extruded erythrocyte showing narrow, joining stalk within wall of capillary.

Fig. 4. Completely extruded erythrocyte with one beginning to follow at same site.

come progressively more and more constricted and their passage correspondingly slower.

When a capillary was caused to contract by gentle mechanical irritation, with a red cell part way through the wall, the cell was frequently pinched in two, the portion inside being carried away by the current, while the portion outside remained on the outer wall and non-hemolyzed, for several hours.

3. *Manipulation of partially extruded erythrocytes.* Erythrocytes, while extravasating, were manipulated with needles in order to secure information regarding the manner of their attachment. The following is a description of three representative experiments on early summer frogs.

a. A capillary was strongly rubbed. This resulted in a partial extrusion of an erythrocyte which took on the shape of a dumbbell almost completely constricted in two. The extruded portion of the corpuscle was punctured, whereupon it faded immediately, while the portion of the cell on the inside of the capillary rounded off, fell away from the wall and was carried off by the blood stream. This suggests the lack of adhesiveness between the cell and the surface of the capillary.

b. The wall of a dilated capillary was irritated until an erythrocyte became partially extruded. Careful stretching and relaxing of the endothelial wall with two microneedles, one on each side of the protruding cell, caused the cell to slip smoothly in and out of the blood vessel, as through a pore, with no indication of adhesiveness.

c. A partially contracted capillary was mechanically irritated. It dilated and, within three minutes, one erythrocyte became completely extravasated, followed by the partial extravasation of a second erythrocyte at the same site in the endothelial wall. The completely extravasated erythrocyte, while in close contact with the protruding part of the second cell, was punctured. It hemolyzed and disappeared. Immediately thereafter, the second erythrocyte rapidly extravasated into the position previously occupied by the cell which had been punctured. A few seconds later, during which the second extravasated cell could be seen oscillating with the pulsation of the heart beat, a third erythrocyte began to pass through at the site of extrusion of the first two cells.

4. *Extravasation time of blood cells.* a. *Leucocytes.* A summary of records is given in the accompanying table of the time taken for the diapedesis of individual leucocytes. The rate was found to vary with the dilated state of the capillary and with the intensity of irritation which induced the reaction.

The passage of a leucocyte through an apparently unstimulated capillary was only occasionally seen. Only three such records were obtained and are given in the first two rows in the table. The remaining two rows present records of diapedesis induced by irritating the capillary. Diapede-

sis by chemical irritation was induced by depositing a droplet, about 0.05 mm. in diameter, of 5 per cent glacial acetic acid in olive oil at a distance from the capillary approximating its diameter.

The actual passage time found in these experiments agrees closely with that reported by Sandison (1931) as being 3 minutes for diapedesis from the capillary in the rabbit's ear. Tannenberg (1925) reported the diapedesis time of leucocytes in the mesentery of the rabbit to be between 5 and 30 minutes.

With more severe irritation, short of rupturing the wall of the capillary, the time for diapedesis of leucocytes may be less than a second.

b. *Erythrocytes.* With severe irritation erythrocytes frequently become attached to the wall of the capillary and then pass through at about the same speed as the leucocytes. A mechanical irritation, sufficient to cause many leucocytes to adhere to the wall, resulted in a transient attachment of erythrocytes. By increasing the irritation an occasional erythrocyte began to be extruded and, with still greater irritation it slowly passed entirely through the wall. This usually took place in the course of several

TABLE

Adhesion and diapedesis time of leucocytes in the mesenteric blood capillaries of the frog

STATE OF CAPILLARY	TYPE OF IRRITATION	AVERAGE LENGTH OF TIME OF			NUMBER OF OBSERVATIONS
		Adhesion	Passage	Total	
		min.	*min.*	*min.*	
Partly contracted	None	24	3	27	2
Dilated	None	17	3	20	1
Dilated	Application of acid	6–9	1–3	7–12	18
Dilated	Mechanical	4–8	2	6–10	7

hours while, with decidedly rough agitation of the capillary, it sometimes required only a few minutes or even seconds. Under the latter condition several cells have been observed to go through the endothelium one after the other at the same spot. When this degree of irritation is present, stasis usually results.

5. *Stasis.* Mechanical or chemical irritation, if sufficiently intense, will cause stasis, viz., a heavy packing of white and red cells which fills the lumen of the capillary and interrupts blood flow. In a typical case a capillary was rubbed vigorously with the tip of a microneedle by moving the needle back and forth against the capillary wall without in any way tearing or piercing the wall. Leucocytes very soon began to adhere to the wall in the region of the irritation. With the progressive piling up of leucocytes erythrocytes were included in the mass of cells which eventually blocked the channel. During this period leucocytes were seldom, if ever, observed to be extravasating.

A similar reaction could be produced by placing a drop of oil saturated with acetic acid close to the capillary.

6. *Recovery.* When the mechanical irritation is insufficient to cause stasis but sufficient to induce red cells in addition to leucocytes to become attached to the wall, normal conditions in the capillary may return at any time within 1 to 60 minutes. Erythrocytes which are not undergoing diapedesis tend to be the first to drop away from the irritated region. These, later, are followed by the leucocytes, some migrating through the wall, others becoming detached and carried away by the blood stream.

Stasis, produced by severe irritation, may not disappear for hours. With lesser irritation it sometimes may disappear within a minute, especially when the cells are not tightly packed. The resolution of stasis and the back and forth pulsation of the plug during the process have been described by Florey (1925) and Landis (1927).

In some cases of recovery from severe stasis, the erythrocytes may remain for hours partially extruded or clinging to the inner wall of the capillary. This condition tends to occur more readily in a capillary at its venous rather than at its arterial end and indicates the greater susceptibility of the venous end to diapedesis. Cohnheim (1889) and Tannenberg (1925) described a similar phenomenon for leucocytes.

Discussion. The evidence presented in this paper indicates that the attachment of the erythrocytes is due not to adhesiveness but to their being caught in small spaces caused by a retraction of the adjacent margins of the endothelial cells. Normally the spaces develop beneath attached leucocytes and close up as the leucocyte passes through. In cases of increased irritation, especially when the capillaries become distended these spaces may persist for a protracted time. They may also appear spontaneously on the wall. It is these spaces not covered by leucocytes which permit the passage of the erythrocytes. These apertures are very small but allow a sufficient outflow of current to carry blood cells to the openings which become plugged as the corpuscles are forced into and through them by the internal capillary pressure.

Upon recovery of the capillary the spaces close up and corpuscles, which happen to be part way through, may either slip back or be forced entirely out of the capillary. Occasionally, the closing of the space may pinch a corpuscle in two. This has not been observed in the case of leucocytes which, apparently, possess the faculty of preventing the adjacent endothelial cells from closing down until its pseudopodium carries the leucocyte all the way through.

Normally, the formation of temporary spaces between the endothelial cells does not cause undue leakage of the capillary. In the case of leucocytic diapedesis none need occur since the spaces through which the leucocytes pass are formed while the latter lie directly on the endothelium. More-

over, their advancing pseudopodia completely fill the opening which usually closes down as the leucocytes pass through. On the other hand, the openings into which the erythrocytes pass appear in the irritated capillary before the erythrocytes touch the wall. However, the consequent outward flow of blood is quickly arrested by the rapidity with which these openings become plugged with erythrocytes.

Our results, with certain modifications, agree with the early views of Cohnheim (1867), Needham (1874) and especially of Arnold (1875, 1878) as opposed to the later ones of Cohnheim (1889) and Krogh (1929) who discarded the idea of interendothelial stomata. Herzog (1925) has also reported indications of holes appearing in the capillary wall. Moreover, our results that the leucocytes and erythrocytes differ radically in the manner of their diapedesis confirm the views of Tannenberg (1925). Tannenberg, who made his observations on the mesentery of the rabbit, states that the diapedesis of red cells depends upon increased blood pressure while that of leucocytes does not.

The migration of leucocytes appears to consist of two stages: first, the adhesive stage, which is protracted when the inducing irritation is moderate, and of short duration when the irritation is severe; second, the actual passage time through the endothelium, which is approximately constant in time under varying degrees of irritation short of that which induces extravasation of erythrocytes. The diapedesis of the leucocyte occurs largely by the directed flow of its own pseudopodium, while that of the erythrocyte is passive and depends upon capillary blood pressure.

Even the leucocytes, at least after they have passed more than half way through the wall, apparently are aided in their passage by the pressure exerted within the capillary. This increase in the rate of extrusion of blood cells in conjunction with an increase in the degree of irritation shows a relationship to the higher pressure ordinarily present in dilated capillaries demonstrated by Landis (1926).

SUMMARY

1. The micromanipulative method was used to irritate one or several endothelial cells of the blood capillary wall. In no case was the wall ever torn.

2. Leucocytes become markedly adhesive after momentarily making contact with an irritated region of the capillary or with leucocytes already adhering there. This reaction may cause the leucocytes to pile up and temporarily occlude the capillary lumen. The tendency of leucocytes to adhere is temporary.

3. The adhesion time of leucocytes, preceding diapedesis, may vary from a few minutes to over half an hour, the period decreasing with increase of irritation and dilatation of the capillary.

4. The actual diapedesis time for leucocytes is approximately 1 to 3 minutes for normal and for moderately irritated capillaries. When the irritation is sufficient to cause extravasation of erythrocytes, the time may decrease to less than one second.

5. Erythrocytes exhibit no adhesive tendencies while attached to or in contact with the wall. This has been demonstrated by manipulating with microneedles.

6. Extravasation of erythrocytes is always accelerated in proportion to the severity of the irritation. With the recovery of the capillary, erythrocytes which are partially extruded may slip back into the blood stream, may pass completely out of the vessel, or may be pinched in two.

7. The indications are that the application of erythrocytes to an irritated wall of the capillary is due to the flow of blood plasma through temporarily induced holes in the endothelial wall. The erythrocytes, caught in the current, become wedged in the holes and the intracapillary pressure forces them through. The leucocytes become attached by the development of an adhesiveness between the wall and the leucocyte. After the leucocyte has once begun to migrate through the wall, its further movements may be assisted by pressure within the capillary.

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PLATELETS AND THE STRUCTURE AND PHYSICAL PROPERTIES OF BLOOD CLOTS

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Platelet-free clots show less firmness, rigidity and contractility than platelet-rich clots. A simple method of demonstrating these differences consists in comparing a clot of blood having a normal number of platelets with one of very low platelet content. The thrombopenic clot is heavier, softer, more jelly-like, more easily displaced and fractured, does not retain the shape of the vessel when removed from it and expresses much less serum than the normal blood clot (fig. 1).

The blood clot is a gel of the swelling, elastic type. The pattern of most gels consists of a three-dimensional solid felt-work of fibrils joined at their points of intersection and with the interspaces filled with fluid (1). On the physical character of this supporting framework depend many of the properties of gels. The mammalian blood clot is a crystalline gel (2) made up of a meshwork of fibrin needles between which is enclosed the liquid phase. This study is devoted to an analysis of the manner in which platelets modify this structure and affect its physical properties.

METHODS. Venous blood from normal and thrombopenic men and dogs and from individuals with hemophilia was collected and, if plasma was required, centrifuged. Paraffin coated vessels and oiled syringes and needles were employed. By adjusting the period and speed of centrifugation, platelet-rich or platelet-free plasma could be obtained. A drop of the blood or plasma was placed on a clean coverslip, ringed with vaseline and inverted over a "hanging drop" slide. Normal blood and plasma were examined, either in their natural state or citrated and diluted. If recalcification was necessary, a small drop of 1 per cent $CaCl_2$ (enough to bring about coagulation) was placed on the coverslip near the drop of blood or plasma and a small isthmus formed between the drops; the preparation was then slightly tilted once to one side. When thus handled and the amount of $CaCl_2$ properly adjusted, the plasma coagulated very slowly from the calcium end to the other.

A field was selected and observed with low and high power objectives and a Zeiss photographic eyepiece, photographs being taken at various

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intervals during coagulation. By a comparative analysis of the serial photographs and observation of the changes taking place in various portions of the plasma, it was possible to determine the differences in the development of the inner structure of the various clots.

Blood of slow coagulation like that from hemophilic individuals offered the best opportunity to observe the movements of the platelets. The coagulation of hemophilic blood, as seen with the dark field microscope differs only from that of normal blood in that it is slower and the fibrin is deposited in thicker, longer, and more widely separated needles (4). The process

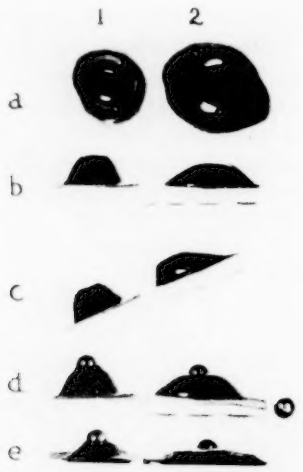

Fig. 1. Various aspects of two blood clots: 1, from a normal dog; 2, from a dog with thrombopenic purpura. *a*, viewed from above; *b*, lateral view; *c*, on an inclined plane; *d*, with a steel ball (weight 1.5 gm.) resting on the clots; *e*, 24 hours after the ball had been placed. The clots were formed in identical, conical shaped porcelain vessels and each was derived from 8 cc. of blood.

thus slowed up and magnified may be easily observed and photographed with the ordinary microscope, unfolding what is essentially, perhaps, a slow motion picture of normal coagulation.

Coagulation of platelet-rich hemophilic plasma. About one-half hour after the preparation was made fibrin needles began to appear. They were most numerous at first at the margin of the drop; among them, there were isolated clumps of lysed platelets. The changes at the margin differed from those toward the center; there was often a thick fibrin network at the periphery of the drop long before the center showed any changes. When the fibrin appeared in the interior of the clot it showed generally, at first, very little relation to the platelets. Soon, however, the platelets slowly

gravitated toward the fibrin needles; eventually nearly every platelet was attached to them, in a manner resembling rain drops on a telegraph wire (fig. 3, a). If the fibrin happened to form in an area where platelets were numerous, the entire shaft of the needle, almost as soon as it appeared, would become covered with them; they were especially numerous at intersections of the fibrin. For a time each individual platelet could be singled out in this loose grouping but gradually fusion took place until fibrin and platelets were united in a solid mass (fig. 3, b). As the platelet groups fused, their outlines were rendered indistinct and the needles became twisted, bent, and more closely knit (fig. 3, c). A square-shaped

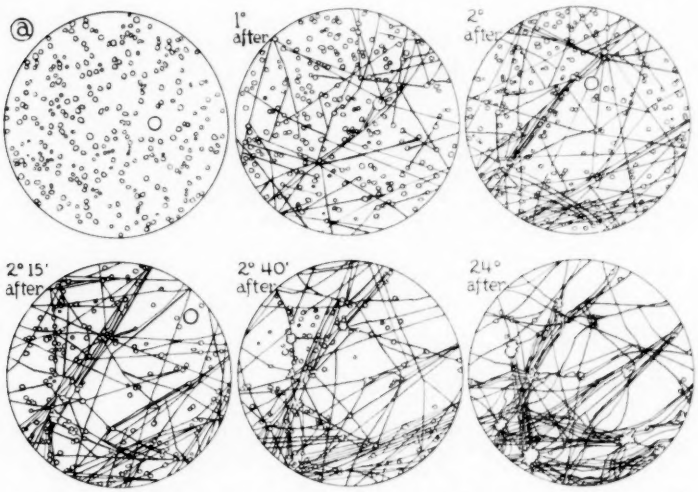

Fig. 2. Drawings made from photographs of the same field in the center of the drop (×200).

area, bounded by fibrin needles, photographed, marked out and measured, diminished in size after the bending of the needles took place. The resulting structure consisted of a mass of intertwining, twisted, knotted *threads* (fig. 3, d). These various changes in their proper time relationships are illustrated in figure 2. After clumping of the platelets around the fibrin, there appeared about them, for a short time, groups of brown, highly refractile globules. These globules correspond perhaps to the vesicles seen, with the aid of the dark field microscope, to emerge from disintegrating platelets (3) (4).

Coagulation of platelet-free hemophilic plasma. The field was optically

empty for approximately three hours after the preparation was made. Then fibrin began to appear in needles approximately twice as long and thick as those from platelet-rich plasma. Gradually they formed a thick meshwork. The majority of them did not fuse and even when close to each other their interstices often remained intact for 3 to 5 days (fig. 3, e). Then, widely separated centers of fusion began to appear, apparently as a result of simple aggregation of the needles (fig. 3, f). It was about these centers that bending and slackening of some of the fibrin were first noted.

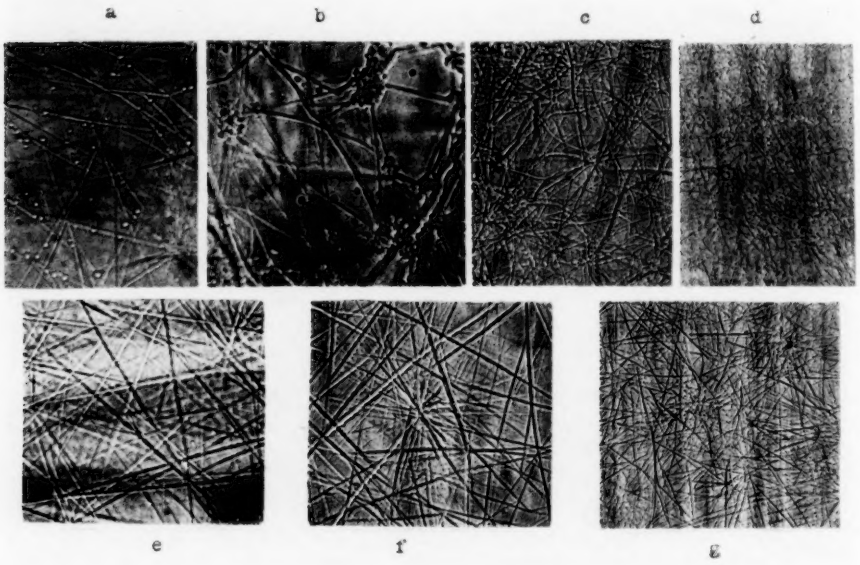

Fig. 3. Aspects of the interior of coagulating platelet-rich (*a*, *b*, *c*, *d*) and platelet-free (*e*, *f*, *g*) hemophilic plasma. *a*, one hour after the beginning of coagulation (×268); *b*, four hours after (×603); *c*, twenty-four hours after (×268); *d*, same as *c* (×67); *e*, three days after the beginning of coagulation (×268); *f*, five days after (×268); *g*, same as *f*, (×67).

The resulting structure consisted mostly of a mass of long, separate *needles* (fig. 3, g).

The contrast between the type of fibrin formed in the neighborhood of platelets and that formed away from them is striking (fig. 3, d, g) and may sometimes be observed in different planes of the same drop of platelet-rich plasma. When the coagulation of hemophilic plasma is unusually slow, the platelets gradually settle and accumulate in the lower half of the drop before fibrin begins to appear. After coagulation is well on its way,

the fibrin in the upper half of the clot is in the form of long, separate *needles* and in the lower half in thinner, twisted, closely packed, knotted *threads*.

Recalcified citrated normal plasma offered a fair opportunity to observe the formation of platelet knots about the fibrin. Towards the center of the mass the platelets clearly showed a tendency to converge to the fibrin needles and particularly to their intersections. The resulting arrangement was similar to that of platelet-rich hemophilic plasma. In rapidly coagulating plasma the fibrin was deposited in short, thin needles, difficult to resolve with transmitted light but easily seen with dark field illumination. The actual formation of the clumps was rarely observed partly because of the rapidity of the coagulation and partly due to the fact that dark-field, oil-immersion lens preparations require a thin, flat glass slide and coverslip.

Fig. 4. For explanation see text

Thus, a film instead of a drop of plasma is examined; pressure or tension exerted through the coverslip influences the behavior of the platelets and alters the arrangement of the fibrin.

When antiplatelet serum was added to normal or slow coagulating platelet-rich plasma, the platelets were agglutinated and lysed in the first few minutes. When fibrin appeared most of the platelets were already in clumps which were later found scattered between and seldom along the needles. Fibrin contact knots at intersections were few and small. As in platelet-free clots the center differed very little in arrangement from the margins. The fibrin was in long and straight needles which did not bend or slacken for several days.

As an aid in visualizing the physical effects that result from the application of mechanical action similar to that of the platelets, on a structure re-

sembling the framework of a clot, models were constructed in which the fibrin was represented by thin flexible wood fibers ("excelsior"). Five groups were arranged, each consisting of 30 fibers, of approximately equal width, and 30 cm. in length. In the first group the fibers were thrown loosely on each other; in the second group, each was tied to another with a fine string, a total of 30 separate knots; to the third group 60 knots were applied; to the fourth, 120 knots; to the fifth, 180 knots. A glance at the result (fig. 4) discloses that as more knots were applied, the mass underwent a gradual decrease in volume, it became more rigid and compact, the fibers showed more and more bending and the interstices decreased progressively in size.

Discussion. Differences in the physical characteristics between platelet-rich and platelet-free clots result perhaps from the fact that in platelet-rich clots the fibrin is bound together by large firm knots, whereas in platelet-free clots it is mostly in the form of separate needles loosely placed one over the other. The latter structure will offer less resistance to a stress, be more easily displaced and, because the needles are not pulled upon and bent, will retain the fluid in its interspaces longer than one in which they have been early united, bent and brought closer together. The bending and shortening of the fibrin needles resulting from the mechanical action of the platelets added to the natural property of contractility of the fibrin will bring about retraction of the clot even when it is held in a narrow glass vessel, with a large amount of its surface exposed to adhesion.

Previous experiments indicated that: when inducing syneresis, platelets act as physiological units and not through any by-products of their disintegration; modified platelets cannot induce syneresis; the total number of platelets in a given amount of blood may not give an indication of the degree a clot will retract, since a variable number of them are destroyed when the blood is shed; within certain limits, the greater the number of undestroyed platelets, the greater the syneresis (5). These facts harmonize with the view here advanced as to the way platelets influence the structure and retractility of the clot. In order to form clumps about the fibrin, platelets must be intact and capable of agglutination. On the available number of these platelets will depend the number of knots formed and these, in turn, will influence the properties of the clot.

That platelet clumps may be found in coagulating blood with strands of fibrin radiating from them, has been known for some time (6). Such a finding was held as an indication that fibrin arose from platelets. It seems, however, that the convergence of intact platelets to previously formed fibrin largely accounts for the appearances described. This process goes on mostly in the interior of the coagulating mass, which remains relatively fluid while the periphery shows evidence of more advanced clot-

ting. In rapidly coagulating blood this phase is of so short duration that it is nearly always missed by the observer; the fibrin is then thought to have originated from the platelets. Perhaps it is mostly in the periphery of a mass of blood that clumping of platelets occurs before the appearance of fibrin and even there the fibrin is not always in the neighborhood of the clumps. Moreover, abundant fibrin may appear in the presence of only a few or no platelets (7).

The convergence of platelets to fibrin needles may be but a manifestation of their known tendency to concentrate at the interface (8). Tension forces developed at the interface of the fluid portion of the plasma and the fibrin would be particularly evident at intersections of the needles where a large amount of surface is offered; perhaps this accounts for the fact that platelets congregate there in greatest numbers.

SUMMARY

In the coagulation of blood from man and dog, soon after fibrin is laid down, *intact* platelets in the interior of the mass converge toward the fibrin needles, adhere to them and form large knots at their intersections. It appears that it is by thus strengthening the fibrin framework that platelets help to render the clot more rigid, firm and elastic. As the knots are being formed, the fibrin becomes bent, twisted and shortened. It is perhaps *while* this is going on and partly as a result of it, that the clot undergoes the visible reduction in volume (syneresis).

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